The Associations between Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, Dietary Intake Patterns and Body Composition

by

Yu Qing Low
(BSc Melb, GDipPsych Deakin)

Submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

Deakin University

May, 2017
I am the author of the thesis entitled

The Associations between Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, Dietary Intake Patterns and Body Composition submitted for the degree of Doctor of Philosophy

This thesis may be made available for consultation, loan and limited copying in accordance with the Copyright Act 1968.

'I certify that I am the student named below and that the information provided in the form is correct'

Full Name: Yu Qing Low

Signed: [Signature Redacted by Library]

Date: 14/08/2017
I certify the following about the thesis entitled
The Associations between Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, Dietary Intake Patterns and Body Composition submitted for the degree of Doctor of Philosophy

a. I am the creator of all or part of the whole work(s) (including content and layout) and that where reference is made to the work of others, due acknowledgment is given.

b. The work(s) are not in any way a violation or infringement of any copyright, trademark, patent, or other rights whatsoever of any person.

c. That if the work(s) have been commissioned, sponsored or supported by any organisation, I have fulfilled all of the obligations required by such contract or agreement.

d. That any material in the thesis which has been accepted for a degree or diploma by any university or institution is identified in the text.

e. All research integrity requirements have been complied with.

'I certify that I am the student named below and that the information provided in the form is correct'

Full Name: Yu Qing Low

Signed: [Signature Redacted by Library]

Date: 19/05/2017
I only hope that we don’t lose sight of one thing – that it all started with a mouse

Walt Disney
ACKNOWLEDGEMENTS

At this point, I would like to sincerely thank the lovely people that have supported me during the work for this thesis. My first and foremost thank you goes to my amazing supervisors, Professor Russell Keast, Dr Kathleen Lacy, and Dr Robert McBride. Thank you for everything. I believe that I am tremendously fortunate to have worked with three outstanding and brilliant individuals, and am deeply appreciative to each of them. Russell, thank you for pushing me to do my best when I feel like I cannot do it. I am so proud of all the challenges that I have overcome but I owe a lot of that to you. I sincerely thank you for all the opportunities you have given to me, sensory expertise, and for tirelessly providing me with invaluable guidance and speedy feedbacks (most often the first email I read the next morning – I have no idea how you did it!). Katie, thank you for all your guidance ever since my psychology years, and for giving me a realistic view of all the pros and cons (mostly cons) of pursuing a PhD. I genuinely thank you for making sure that I talked to a career counsellor before I made up my mind, for without it (she made me draw a huge mind map!), I would have already given up halfway through. Thank you for all your nutrition expertise and most importantly for guiding me as a friend. I am greatly indebted to Dr Robert McBride, Adjunct Associate Professor at Deakin University, who sadly passed away before this thesis was submitted. Rob, while seriously ill, read through two of my final manuscript drafts, and gave many insightful criticisms and suggestions for improvement. Your death is a great personal loss to many of us, and also a huge loss to the world of taste psychophysics. I am very fortunate to have been able to refer to you as my supervisor, and to learn so much from you and Russell when developing methods to assess oral complex carbohydrate sensitivity. Thank you for all your encouraging words, and I am
pleased to know that you were satisfied with my progress in the last email you sent to me. You will be sadly missed.

I would also like to thank all the amazing CASS members for all your support throughout this journey. Gie and Ramon – thank you for all your guidance and help. Sara – thank you for all your positive encouragement, guidance, and chats. Megan - thank you for all your hugs, Disney, babies (doggy), and #YOLO chats. Thank you also to the school’s lab, admin, and finance staff for all your assistance. Sandra – thank you for helping me buy all the low fat creams within the 5km radius for my milkshake study. A special thank you to Uracha, Andrew, Carmen, and Inga #mysuperhelpers for helping me out for the milkshake study.

I wish to express a sincere thank you to all my participants, many of whom were friends, family, and Deakin staff who so graciously agreed to participate in my studies. Without them, the completion of this thesis would not have been possible.

I would also like to thank all of my wonderful Deakin friends for the fun times we have shared during this journey. Firstly, to Penny and Sim – thank you. Penny, thank you so much for your friendship, support, guidance about anything sensory related, excel and power points tips, laughter, shopping tips, and gossips that we have shared in the past years. I am going to miss how we used to only travel five stars together, our Chai times (they do not taste as good anymore since you left), and your very picky eating habits. Sim, thank you for all your help with Photoshop, presentation, and poster designs, YSL and Chanel talks, concerns, and friendship. Thank you for always offering to send me home when it’s getting late, and for sending me hot chocolates and Nutella bars. Most importantly, thank you for always having my back.
To the lovely crew (past and present) of J4.39 #BESTOFFICEEVER Meagan, Sheena, Penny, Madi, Sim, Kelsey, Andrew, Kathryn, and Dong Li – thank you for all the fun times and endless laughs all over the years! I super duper appreciate all the support from each and every one of you, inside and outside of the office. I am going to miss the random 90s sing-a-long sessions on Fridays, movie/Friends marathon sessions while labelling cups, birthday surprises, and long walks. Madi – thank you for your friendship and most of all thank you for making Mondays so interesting after your weekend fun. Kelsey – thank you for being so entertaining with your sarcasm (although I don’t get it most of the time). Andrew – thanks for sharing your geeky side (Nintendo, consoles, and technologies) and just always being there to talk to. Thank you all for being amazing office buddies and friends! Thanks to our rivalry office, J4.46 (past and present) - Peggy, Rivkeh, Karen, Lisa, Manuela, Uracha, Ajam, Inga, and Jaana for keeping up with my rants and for always checking up on me (this truly has helped me get through my PhD and I appreciate it) over the past few years. Jen and Linda – you weren’t in my office, but thank you for being there and for being a friend.

I would also like to express my gratitude and appreciation to my close circle of friends/‘family’ outside Deakin (My Melbourne ‘Family’ - Trinity, Melbourne, Psych mates, Tuna, Coconut) for all your encouragement and support. Szen - thank you for all your help in labelling the 30k cups and all your positive encouragement! Fransisca (nenek) – thank you for always listening to me whine and cry this whole time. Thank you all for always being there. I am so lucky to have such amazing and caring friends. You guys are definitely my surrogate family.
The biggest thank you to my parents, for giving me the world. Thank you for your previous gift of education in Australia – of which I am eternally grateful for. All the love and support that you have provided me over the years was the greatest gift anyone has ever given to me, and thank you for allowing me to realise my own dreams and potential (and not forcing me to study any commerce degree). Thank you for giving me the opportunity to learn to be independent at a very young age of 18 in a land far away from home, and for always calling and messaging me to make sure that I am taking enough breaks. I am forever in debt for your love and guidance. Thank you. Thank you also to my only brother, Billy for all your love and unremitting encouragement over the years. My future in laws, Darren, Ying and Sophia deserve a special mention because of all their unrelenting support during this time.

I would like to thank my new little family. My fiancé, Damien – thank you and I love you. There are no words to say how grateful I am for all your love, support, and patience in the past 10 years. We can now finally prepare for our wedding. My beautiful babies, Bubu Armageddon Kong and Chacha Pomegranate Kong – thank you for always being my number one fans. I have spent most of my time writing this thesis with the three of you (and also Billy). I love all of you to the moon and back.

Last but not least, thank you to my sources of caffeine (coffee, diet coke) and dopamine (chocolates) for keeping me going. And to you reading this right now. Thank you.
PUBLICATIONS AND AWARDS FROM THIS THESIS

Manuscripts published:


Manuscripts submitted:


Low JY, Lacy KE, McBride R, Keast RS. The Associations between Oral Complex Carbohydrate Sensitivity, Anthropometry, and Dietary Intake in Adults. Submitted
April 2017, currently under review at *Journal of Nutrition. Data presented in Chapter Six.*

**Conference abstracts:**

**The 8th Annual Australia and New Zealand Sensory & Consumer Science Symposium, Melbourne, Australia**


**The 47th Australian Institute of Food Science and Technology (AIFST) Annual Convention, Melbourne, Australia**


**The Australasian Association for ChemoSensory Sciences Conference (AACSS), Brisbane, Australia**

The Australian Institute of Food Science and Technology (AIFST) Summer School, Melbourne, Australia


The 11th Pangborn Sensory Science Symposium, Gothenburg, Sweden


The 17th International Symposium on Olfaction and Taste (ISOT2016), Yokohama, Japan


The 49th Australian Institute of Food Science and Technology (AIFST) Annual Convention, Brisbane, Australia

The 50th Australian Institute of Food Science and Technology (AIFST) Annual Convention, Sydney, Australia


The 12th Pangborn Sensory Science Symposium, Providence, USA


**Awards:**

Australian Institute of Food Science & Technology (AIFST) Malcolm Bird Award 2017 Winner, 17th July 2017, Sydney, Australia.

The Rick Bell Memorial Travel Scholarship to attend the 2017 Pangborn Sensory Science Symposium in Providence, Rhode Island, USA.

Young Investigator Award, 17th International Symposium on Olfaction and Taste (ISOT2016), 5th June 2016-9th June 2016, Yokohama, Japan.
Australian Institute of Food Science & Technology (AIFST) Sensory Solutions

Tony Williams Sensory Award 2016 Winner, 28th June 2016, Brisbane, Australia.

School of Exercise and Nutrition Sciences Honorable Mention Award in the Park Room at the Research Degree Symposium 2014, 5th September 2014, Melbourne, Australia.

The People’s Choice Award in the School of Exercise and Nutrition Sciences’ Three Minute Thesis Competition, Second Place, 13th June 2014, Melbourne, Australia.
TABLE OF CONTENTS

ABSTRACT ........................................................................................................................... I

LIST OF TABLES................................................................................................................. XII

LIST OF FIGURES.............................................................................................................. XVII

LIST OF APPENDICES....................................................................................................... XXI

LIST OF ABBREVIATIONS................................................................................................. XXIII

CHAPTER ONE: LITERATURE REVIEW1 ........................................................................... 1

1.1 Introduction .................................................................................................................. 1

1.2 The Role of Sweet Taste in Satiation and Satiety ....................................................... 3
  1.2.1 Overweight/Obesity ............................................................................................... 3
  1.2.2 Taste and Its Function .......................................................................................... 5
  1.2.3 Peripheral and Central Mechanisms for Sweet Taste Detection ....................... 9
    1.2.3.1 Peripheral Mechanisms for Sweet Taste Detection ....................................... 10
    1.2.3.2 Sweet Taste Detection in the Oral Cavity .................................................... 12
    1.2.3.3 Sweet Taste Detection in the Gastrointestinal Tract ..................................... 13
    1.2.3.4 Non-Nutritive Sweeteners and Their Role in the Gastrointestinal Tract .................. 14
    1.2.3.5 Sweet Taste Processing in the Brain ................................................................. 16
    1.2.3.6 Are Non-Nutritive Sweeteners Equally as Rewarding to the Brain as Caloric Sugars? ............................................................... 17
  1.2.4 Possible Role of Sweet Taste Function ................................................................. 18
    1.2.4.1 Individual Differences in Sweet Taste Function .......................................... 18
  1.2.5 Implications of Decreased Sweet Taste Function, the Gastrointestinal System, and Satiety ................................................................. 22
    1.2.5.1 Implications of Sweet Taste Function on Body Mass Index ......................... 22
    1.2.5.2 Sweet Taste Appetite ....................................................................................... 23
    1.2.5.3 Links between Sweet Taste Perception of Caloric Sweeteners and Non-Nutritive Sweeteners and Satiation ........................................... 24
    1.2.5.4 Links between Attenuated Sweet Taste Perception, the Gastrointestinal System, and Satiety ................................................................. 27
  1.2.6 Conclusions – The Role of Sweet Taste in Satiation and Satiety ......................... 27

1.3 The Evidence Supporting the Existence of Oral Complex Carbohydrate Sensitivity .................................................................................................................. 29
  1.3.1 Taste Criteria and Terminology ............................................................................. 29
  1.3.2 Criterion One: Provides an Evolutionary Advantage ........................................ 31
  1.3.3 Criterion Two: Elicited by a Unique Class of Chemical ...................................... 31
1.3.4 Criterion Three: Independent Transduction Mechanism ........................................ 32
  1.3.4.1 Unidentified Complex Carbohydrate ‘Taste’ Receptor .................................... 33
  1.3.4.2 T1R-Independent Sweet Sensing Pathways ...................................................... 35
1.3.5 Criterion Four: Signals are Detected through Gustatory Nerves that are Processed in the Gustatory Centres ................................................................................ 36
1.3.6 Criterion Five: Perceptual Independence .................................................................. 36
1.3.7 Criterion Six: Behavioural and/or Physiological Responses to Oral Complex Carbohydrate Exposure .......................................................................................................................... 37
1.3.8 Possible Functions of Oral Sensitivity to Complex Carbohydrates ......................... 38
1.3.9 Conclusions – The Evidence Supporting the Existence of Oral Complex Carbohydrate Sensitivity .......................................................................................................................... 39

1.4 Thesis aim, objectives, and hypotheses ........................................................................ 43
  1.4.1 Overall Aim and Objectives .................................................................................. 43
  1.4.2 Hypotheses ........................................................................................................... 44

CHAPTER TWO: MATERIALS, METHODOLOGY, AND MEASUREMENTS^1 .......................................................................................................................... 46

2.1 Introduction .................................................................................................................. 46

2.2 Participants .................................................................................................................. 46
  2.2.1 Participant Demographics ................................................................................... 46
  2.2.2 Reimbursement .................................................................................................... 47

2.3 Ethics ............................................................................................................................ 47

2.4 Sensory Testing ............................................................................................................ 48
  2.4.1 Sensory Testing .................................................................................................... 48
  2.4.2 Sweet Taste Solutions – Materials and Methods .................................................. 48
  2.4.3 Sweet Taste Solutions – Threshold Measurements .............................................. 50
  2.4.4 Sweet Taste Solutions – Intensity Measurements ............................................... 56
    2.4.4.1 Participant Training ....................................................................................... 56
    2.4.4.2 Standardisation of gLMS Usage with Weight Ratings .................................. 57
    2.4.4.3 Sweet Taste Intensity Measurement Procedures .......................................... 58
  2.4.5 Oral Complex Carbohydrate Sensitivity Solutions – Materials and Methods ......... 58
    2.4.5.1 Analysis of Common Sugars in Maltodextrin and Oligofructose Samples .. 61
  2.4.6 Oral Complex Carbohydrate Sensitivity Solutions – Threshold Measurements .... 62
  2.4.7 Oral Complex Carbohydrate Sensitivity Solutions – Intensity Measurements ....... 63
    2.4.7.1 Participant Training and Standardisation of gLMS with Weight Ratings ........ 63
2.4.7.2 Oral Complex Carbohydrate Sensitivity - Intensity Measurement

Procedures ........................................................................................................... 63

2.4.8 Five Primary Tastes – Materials and Methods .............................................. 64

2.4.9 Five Primary Tastes – Threshold Measurements........................................... 67

2.4.10 Five Primary Tastes – Intensity Measurements........................................... 68

2.4.10.1 Participant Training and Standardisation of gLMS with Weight Ratings

............................................................................................................................. 68

2.4.10.2 Five Primary Tastes - Intensity Measurement Procedures.................... 68

2.4.11 Hedonic Ratings – Materials and Methods.............................................. 69

2.4.12 Hedonic Ratings – Measurements ............................................................... 71

2.4.12.1 Standardisation of Hedonic Scale Usage with Non-Food Items........... 71

2.5 Body Composition .............................................................................................. 72

2.6 Dietary Intake ..................................................................................................... 73

2.6.1 Dietary Questionnaire for Epidemiological Studies Version 2 ..................... 73

2.6.2 Deakin Food Frequency Questionnaire ......................................................... 74

2.6.3 Diet Diary ...................................................................................................... 74

2.6.4 Non-Nutritive Sweetener Consumption ....................................................... 75

2.7 Three-Factor Eating Questionnaire................................................................... 76

2.8 Satiation Measures - Preload and Ad Libitum Intake of Milkshakes,

Drinking Rate, Appetite, and Hedonic Ratings....................................................... 76

2.8.1 Satiation Measures - Materials ...................................................................... 76

2.8.2 Satiation Measures – Methods and Measurements........................................ 77

CHAPTER THREE: STUDY 1(A) PSYCHOPHYSICAL EVALUATION OF
SWEETNESS FUNCTIONS ACROSS MULTIPLE SWEETENERS1........... 80

3.1 Introduction ........................................................................................................ 80

3.2 Aims and Hypotheses ......................................................................................... 83

3.2.1 Aims ............................................................................................................... 83

3.2.2 Hypotheses ..................................................................................................... 83

3.3 Subjects, Materials, and Methods.................................................................... 83

3.3.1 Subjects .......................................................................................................... 83

3.3.2 Study Design .................................................................................................. 84

3.3.3 Participant Training ....................................................................................... 85

3.3.4 Stimuli ............................................................................................................. 85

3.3.5 Detection and Recognition Threshold Determination for the Five Primary
Tastes ...................................................................................................................... 86

3.3.6 Detection and Recognition Threshold Determination for Sweet Taste........ 89

3.3.7 Suprathreshold Intensity Ratings for the Five Prototypical Tastes and
Sweeteners .............................................................................................................. 89
3.3.8 Standardisation of gLMS Usage with Weight Ratings
3.3.9 Statistical Analysis

3.4 Results

3.4.1 Detection and Recognition Thresholds of Sweeteners
3.4.2 Suprathreshold Intensities for Sweeteners
3.4.3 Relationships between Sweet Taste Measures
3.4.4 Detection and Recognition Thresholds of Prototypical Tastants
3.4.5 Suprathreshold Intensities of Prototypical Tastants and Relationship with Detection and Recognition Thresholds
3.4.6 Relationships between Sweet Taste Function and Prototypical Taste Function

3.5 Discussion

3.6 Conclusion

CHAPTER FOUR: STUDY 1(B) THE ASSOCIATIONS BETWEEN SWEET TASTE FUNCTION, BODY COMPOSITION, AND DIETARY INTAKE IN ADULTS

4.1 Introduction

4.2 Aims and Hypotheses

4.2.1 Aims
4.2.2 Hypotheses

4.3 Subjects, Materials, and Methods

4.3.1 Subjects and Study Design
4.3.2 Participant Training
4.3.3 Stimuli
4.3.4 Detection and Recognition Threshold Determination for Sweet Taste and Suprathreshold Intensity Ratings for Sweeteners
4.3.5 Standardisation of gLMS Usage with Weight Ratings
4.3.6 Body Composition
4.3.7 Dietary Intake
4.3.8 Statistical Analysis

4.4 Results

4.4.1 Sweet Taste Function of Sweet Tastants
4.4.2 Sweet Taste Function of Body Composition
4.4.4 Sweet Taste Function and Consumption of Added Sugar and Specific Sugar-Sweetened Foods
4.4.5 Sweet Taste Function and Consumption of Non-Nutritive Sweeteners

4.5 Discussion
4.6 Conclusions ............................................................................................................... 125

CHAPTER FIVE: STUDY 2(A) EVIDENCE SUPPORTING ORAL SENSITIVITY TO COMPLEX CARBOHYDRATES INDEPENDENT OF SWEET TASTE SENSITIVITY IN HUMANS1 ................................................. 126

5.2 Aims, Hypotheses, and Terminologies................................................................. 128
  5.2.1 Aims .................................................................................................................. 128
  5.2.2 Hypotheses ..................................................................................................... 128
  5.2.3 Terminologies .............................................................................................. 129

5.3 Subjects, Materials, and Methods ..................................................................... 129
  5.3.1 Study Design .................................................................................................. 129
  5.3.2 Subjects ......................................................................................................... 131
  5.3.3 Participant training ....................................................................................... 131
  5.3.4 Stimuli ........................................................................................................... 131
  5.3.5 Analysis of Common Sugars in Maltodextrin and Oligofructose Samples. 135
  5.3.6 Detection Threshold Determination for Sweet Taste and Oral Sensitivity to Complex Carbohydrates ............................................................. 135
  5.3.7 Detection Threshold Determination for Salty, Sour, Bitter, and Umami Tastes ................................................................................................................. 137
  5.3.8 Suprathreshold Intensity Ratings for the Sweeteners, Complex Carbohydrates, and Prototypical Tastants .......................................................... 138
  5.3.9 Standardisation of gLMS Usage with Weight Ratings .............................. 138
  5.3.10 Statistical Analysis ..................................................................................... 141

5.4 Results .................................................................................................................. 142
  5.4.1 Test-retest Reliability of Complex Carbohydrates ...................................... 142
  5.4.2 Oral Detection Thresholds of Complex Carbohydrates and Relationship with Taste Detection Thresholds of Sweeteners .............................................. 142
  5.4.3 Suprathreshold Intensities for the Complex Carbohydrates and Relationship with Suprathreshold Intensities for Sweeteners ........................................... 147
  5.4.4 Relationships between Oral Detection Thresholds and Suprathreshold Intensities of Complex Carbohydrate Solutions ............................................. 151
  5.4.5 Taste Function of Prototypical Tastants and Relationship with Oral Detection Thresholds and Suprathreshold Intensities of Complex Carbohydrate Solutions 151
  5.4.6 Relationships between Oral Complex Carbohydrate Sensitivity, Sweet Taste Function, and Prototypical Taste Function ............................................. 152

5.5 Conclusion ......................................................................................................... 163

CHAPTER SIX: STUDY 2(B) THE ASSOCIATIONS BETWEEN ORAL COMPLEX CARBOHYDRATE SENSITIVITY, BODY COMPOSITION, AND DIETARY INTAKE IN ADULTS .......................................................... 164

6.1 Introduction ........................................................................................................... 164
6.2 Aims, Hypotheses, and Terminologies ............................................................ 166
   6.2.1 Aims ............................................................................................................. 166
   6.2.2 Hypotheses ............................................................................................... 166
   6.2.3 Terminologies ........................................................................................... 166

6.3 Subjects, Materials, and methods ..................................................................... 167
   6.3.1 Study Design ............................................................................................... 167
   6.3.2 Subjects ....................................................................................................... 167
   6.3.3 Participant Training ..................................................................................... 168
   6.3.4 Stimuli ......................................................................................................... 168
   6.3.5 Detection Threshold Determination and Suprathreshold Intensity Ratings for Oral Sensitivity to Complex Carbohydrates ......................................................... 168
   6.3.6 Body Composition ..................................................................................... 168
   6.3.7 Dietary Intake ............................................................................................. 169

6.4 Results ................................................................................................................ 173
   6.4.1 Participants ................................................................................................. 173
   6.4.2 Oral Complex Carbohydrate Sensitivity of Maltodextrin and Oligofructose .............................................................................................................................. 175
   6.4.3 Oral Complex Carbohydrate Sensitivity and Body Composition ............ 175
   6.4.4 Oral Complex Carbohydrate Sensitivity and Energy and Macronutrient Intakes ......................................................................................................................... 178
   6.4.5 Associations between Body Composition Measurements and Dietary Intake .............................................................................................................................. 180
   6.4.6 Correlations between Dietary Assessment Tools ....................................... 180

6.5 Discussion .......................................................................................................... 182

6.6 Conclusions ....................................................................................................... 187

CHAPTER SEVEN: STUDY 3(A) THE ASSOCIATIONS BETWEEN ORAL COMPLEX CARBOHYDRATE SENSITIVITY, BMI, LIKING, AND CONSUMPTION OF COMPLEX CARBOHYDRATE BASED FOODS ...... 188

7.1 Introduction ...................................................................................................... 188

7.2 Aims, Hypotheses, and Terminologies ............................................................ 190
   7.2.1 Aims ............................................................................................................. 190
   7.2.2 Hypotheses ............................................................................................... 190
   7.2.3 Terminologies ........................................................................................... 191

7.3 Subjects, Materials, and Methods .................................................................. 192
   7.3.1 Subjects ................................................................................................... 192
   7.3.2 Study Design ............................................................................................. 193
   7.3.3 Participant Training ................................................................................... 196
   7.3.4 Stimuli ....................................................................................................... 196
   7.3.4.1 Analysis of Common Sugars in Maltodextrin Sample ......................... 197
7.3.5 Detection Threshold Determination for Sweet Taste and Oral Sensitivity to Complex Carbohydrates .......................................................... 199
7.3.6 Suprathreshold Intensity Ratings for Glucose and Maltodextrin ................. 200
7.3.7 Standardisation of gLMS Usage with Weight Ratings ............................. 200
7.3.8 Hedonic Ratings for Sweet and Complex Carbohydrate Solutions and Prototypical Foods ................................................................. 202
7.3.9 Standardisation of Hedonic Scale Usage with Non-Food Items .................... 204
7.3.10 Body Composition .............................................................................. 204
7.3.11 Dietary Intake .................................................................................... 205
7.3.12 Statistical analysis ...............................................................

7.4 Results ........................................................................................................ 208
7.4.1 Participants .......................................................................................... 208
7.4.2 Sweet Taste Function and Oral Complex Carbohydrate Sensitivity ........... 208
7.4.3 Relationships between Oral Complex Carbohydrate Sensitivity and Sweet Taste Function ............................................................ 212
7.4.4 Hedonic Ratings for Sweet and Complex Carbohydrate Solutions and Prototypical Foods ............................................................... 214
7.4.5 Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, and Hedonic Ratings for Sweet and Complex Carbohydrate Solutions and Prototypical Foods ........................................................................ 214
7.4.6 Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, and BMI 217
7.4.7 Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, and Frequency of Consumption of Sweet and Complex Carbohydrate Based Foods 217
7.4.8 Liking of Sweet and Complex Carbohydrate Solutions and Prototypical Foods, BMI, and Frequency of Consumption of Sweet and Carbohydrate Based Foods ................................................................. 221

7.5 Discussion .................................................................................................... 224

7.6 Conclusions ................................................................................................. 229

CHAPTER EIGHT: STUDY 3(B) THE ASSOCIATIONS BETWEEN SWEET TASTE FUNCTION, ORAL COMPLEX CARBOHYDRATE SENSITIVITY, LIKING, AND CONSUMPTION OF AD LIBITUM SWEET AND COMPLEX CARBOHYDRATE MILKSHAKES ................................................................. 230

8.1 Introduction .................................................................................................. 230

8.2 Aims, Hypotheses, and Terminologies .......................................................... 233
8.2.1 Aims ................................................................................................... 233
8.2.2 Hypotheses ....................................................................................... 234
8.2.3 Terminologies ................................................................................... 234

8.3 Subjects, Materials, and Methods ................................................................. 234
8.3.1 Subjects ........................................................................................... 234
Abstract

The prevalence of obesity is rising in developed nations and this has been associated with increased energy intake, in particular greater intakes of sweet foods due to their high palatability and wide availability. Sweet foods consumption is regulated by many factors, one of which may be the ability to perceive sweetness. Yet, the role of taste function in promoting consumption of particular nutrients or ingredients related with obesity has long been an area of investigation, but with varied research support. In regards to sweet taste, whether or not environmental influences such as habitual dietary intake can modify sweet taste function or vice versa remains controversial. For example, some reports supported an inverse correlation between body mass index (BMI) and sweet taste function (lower BMIs were correlated with greater sensitivity to sweet taste), while the majority of data showed no link between BMI and sweet taste function. While data are conflicting, it is essential to note that discrepancies between studies may be attributed to the type of psychophysical methodology used, or researchers using only one measure of sweet taste function as each measure of sweet taste function represents a different dimension of the sense of sweet taste. There are three perceptual dimensions of sweet taste function, namely detection threshold, recognition threshold, and suprathreshold intensity perception. It is also probable that the inconsistencies between studies may be attributed to the type of sweetener used, that is, caloric sweeteners and non-nutritive sweeteners. In the past century, new non-nutritive sweeteners have been introduced into our diets. A confounding factor in this area is that non-nutritive sweeteners generally contain only negligible amounts of kilojoules thereby decoupling sweetness from energy value. Questions remain regarding how these non-nutritive sweet compounds relate to caloric
Abstract

sweeteners (i.e. the perceptual relationships between caloric and non-nutritive
sweeteners using a range of psychophysical measures within a single group of
individuals), and the associations between sweet taste function, body composition, and
dietary intake.

Dietary carbohydrates in the form of complex carbohydrates and simple
carbohydrates represent two essential sources of energy in our diet. For most plants,
complex carbohydrates are more abundant than simple carbohydrates, but it is sugars
with their strong palatable sweetness that are the most taste-visible carbohydrates. One
of the many functions of the taste system is to act as a nutrient-toxin detection system;
for example, sweet taste indicates the presence of energy, while bitter taste indicates
the presence of poisons. However, it has been suggested recently that sweet taste
perception or the degree of sweetness in general is not a good proxy for the amount of
energy available in a food. Rather, a detection mechanism encouraging consumption
of complex carbohydrate rich foods independent from sweet taste may be
advantageous to provide quantitative information about food energy content for
physiological functioning. Indeed, there is increasing support from animal studies in
the past decades indicating that rodents and even some non-human primates are
attracted to the taste of complex carbohydrates derived from maltodextrin. It has also
been reported that humans may perceive complex carbohydrates, and that the
sensitivity to simple carbohydrates (glucose, sucrose) was qualitatively distinctive
from complex carbohydrates (maltodextrin, glucose oligomers). While the human taste
perception of complex carbohydrate (starch) hydrolysis products has been well
investigated, it needs replication and also extension. For example, the psychophysics
of oral complex carbohydrate sensitivity and its associations with sweet taste function
across multiple sweeteners has not yet been explored completely. It is important to
note, research that includes taste as a variable generally uses only one measure of taste
Abstract

function or a limited number of concentrations to measure oral sensitivity to complex carbohydrates. Furthermore, it remains to be verified, whether complex carbohydrates have a unique quality that does not overlap with the five prototypical taste primaries (sweet, sour, salty, bitter, and umami), and also if an individual’s sensitivity to complex carbohydrates remains stable over numerous testing sessions.

The latest development in knowledge surrounding sweet taste mechanisms for caloric sweeteners within the mouth, the brain, and the gut (nutrient sensors), along with their fundamental role in appetite regulation, and therefore, dietary energy intake, suggests that abnormalities in any or several of these nutrient sensors may be a key to understanding why some people consume more energy than others. It is therefore important to understand the individual differences in the ability to perceive dietary carbohydrates (both simple and complex carbohydrates), and how these may influence dietary intake (habitual/usual intake) and short-term appetite (satiation/acute intake). Although largely hypothetical at the current time, the associations between sweet taste function, oral complex carbohydrate sensitivity, dietary intake, and satiation suggest a fascinating model to understand drivers of carbohydrate food consumption.

The aim of this thesis was to assess whether individual variations in sweet taste function and oral complex carbohydrate sensitivity may influence body composition and dietary intake (both habitual and acute intake). A secondary aim was to investigate the psychophysics of sweet taste function and oral complex carbohydrate sensitivity across a range of sweet tastants and complex carbohydrates. The objectives of this thesis were: (i) to investigate the three main measures of sweet taste function – detection threshold, recognition threshold, and suprathreshold intensity – across a range of caloric and non-nutritive sweeteners; (ii) to investigate the associations between the three common measures of sweet taste function using multiple sweeteners,
body composition, and dietary intake among adults; (iii) to investigate if humans can perceive two complex carbohydrates including a soluble starch, maltodextrin, and a soluble fibre, oligofructose; (iv) to investigate associations between oral complex carbohydrate sensitivity (maltodextrin, and a soluble fibre oligofructose), body composition, and dietary intake among adults; (v) to confirm if humans can perceive complex carbohydrate from a range of concentration levels; and if oral sensitivity to complex carbohydrate relates to BMI, liking, and consumption of carbohydrate foods in a larger sample group of adults; (vi) and to measure if sweet taste function and oral sensitivity to complex carbohydrates relates to *ad libitum* consumption of sweet and complex carbohydrate milkshakes in a sample group of adults. This thesis included three major studies (each study is presented in two chapters).

**Study 1(a): Psychophysical Evaluation of Sweetness Function Across Multiple Sweeteners**

*This abstract has been published in Chemical Senses (2016): bjw109 as ‘Psychophysical Evaluation of Sweetness Function Across Multiple Sweeteners’*

Sweetness is one of the five-prototypical tastes and is activated by sugars and non-nutritive sweeteners (NNS). The aim of this study was to investigate measures of sweet taste function (detection threshold, recognition threshold, and suprathreshold intensity perception) across multiple sweeteners. Sixty participants, 18-52 years of age (mean age in years = 26, SD = ± 7.8), were recruited to participate in the study. Detection threshold and recognition threshold were collected for caloric sweeteners (glucose, fructose, sucrose, erythritol) and NNS (sucralose, Rebaudioside A). Sweetness intensity for all sweeteners was measured using a general Labeled Magnitude Scale. There were strong correlations between detection threshold and recognition threshold of all four caloric sweeteners across people (*r* = 0.62 – 0.90, *P* < 0.05).
Abstract

0.001), and moderate correlations between detection threshold and recognition threshold for both of the NNS ($r = 0.39–0.48$, $P < 0.05$); however, weaker correlations were observed between the detection threshold and recognition threshold of the caloric sweeteners and NNS ($r = 0.26–0.48$, $P < 0.05$). The detection threshold and recognition threshold of glucose and fructose were not correlated with detection threshold and recognition threshold of sucralose ($P > 0.05$). In contrast, there were strong correlations between the sweetness intensity ratings of all sweeteners ($r = 0.70–0.96$, $P < 0.001$). This suggests those caloric sweeteners and NNS access at least partially independent mechanisms with respect to detection threshold and recognition threshold measures. At suprathreshold intensity perception, however, the strong correlation between caloric sweeteners and NNS through weak, moderate, and strong intensity indicates a commonality in sweet taste mechanism for the perceived intensity range.

Study 1(b): The Associations between Sweet Taste Function, Body Composition, and Dietary Intake in Adults

This abstract has been published in Nutrients, 8(4), 241 as ‘The Association between Sweet Taste Function, Anthropometry, and Dietary Intake in Adults’

Variations in ability to detect, recognise, and perceive sweetness may influence food consumption, and eventually chronic nutrition-related conditions such as overweight and obesity. The aim of this study was to investigate the associations between sweet taste function, body composition, and dietary intake in adults. Participants’ ($n = 60$; mean age in years = 26, SD = ± 7.8) sweet taste function for a range of sweeteners (glucose, fructose, sucrose, sucralose, erythritol, and Rebaudioside A) was assessed by measuring detection threshold, recognition
Abstract

threshold, and suprathreshold intensity perception. Height, weight, and waist circumference were also measured, and participants also completed a Food Frequency Questionnaire. There was large inter-individual variation in sweet taste function measures. Pearson’s correlation coefficient revealed no robust correlations between measures of sweet taste function, body composition, and dietary intake, with the exception of suprathreshold intensity, which was moderately correlated with total energy intake ($r = 0.23–0.40$). One-way analysis of variance revealed no significant differences between the most and least sensitive participants and those who experienced high and low intensity in terms of BMI, waist circumference, and dietary intake for all measures of sweet taste function and sweeteners (all $P > 0.01$). When stratified into BMI categories, there were no significant differences in any measure of sweet taste function between the normal weight and overweight/obese participants (all $P > 0.01$). Results show that sweet taste function is not associated with body composition and sweetness intensity measures are the most appropriate measure when assessing links between sweet taste and food consumption in comparison to threshold measures.

Study 2(a): Evidence Supporting Oral Sensitivity to Complex Carbohydrates Independent of Sweet Taste Sensitivity in Humans

This abstract is currently under review at Plos One Journal as ‘Evidence Supporting Oral Sensitivity to Complex Carbohydrates Independent of Sweet Taste Sensitivity in Humans’

Compared to simple sugars, complex carbohydrates have been assumed invisible to taste. However, two recent studies proposed that there may be a perceivable taste quality elicited by complex carbohydrates independent of sweet taste.
Abstract

There is precedent with behavioural studies demonstrating that rats are very attracted to complex carbohydrates, and that complex carbohydrates are preferred to simple sugars at low concentrations. This suggests that rats may have independent taste sensors for simple sugars and complex carbohydrates. The aim of this study is to investigate detection threshold and suprathreshold intensity for two complex carbohydrates, maltodextrin and oligofructose, and six sweeteners, glucose, fructose, sucrose, sucralose, Rebaudioside A, and erythritol. There were strong correlations between the detection thresholds and mean intensity ratings for complex carbohydrates (maltodextrin, oligofructose) ($r = 0.94, P < 0.001$). There were no significant correlations between the detection thresholds of the complex carbohydrates (maltodextrin, oligofructose) and the sweeteners (glucose, fructose, sucralose, Rebaudioside A, and erythritol) (all $P > 0.05$). However, moderate correlations were observed between perceived intensities of complex carbohydrates and sweeteners ($r = 0.48-0.61, P < 0.05$). These data provide evidence that complex carbohydrates can be sensed in the oral cavity over a range of concentrations independent of sweet taste sensitivity at low concentration levels, but with partial overlap with sweet taste intensity at higher concentrations.

Study 2(b): The Associations between Oral Complex Carbohydrate Sensitivity, Body Composition, and Dietary Intake in Adults

This abstract is currently under review at Journal of Nutrition as ‘The Associations between Oral Complex Carbohydrate Sensitivity, Anthropometry, and Dietary Intake in Adults’
Compared to simple carbohydrates, complex carbohydrates have been assumed invisible to taste. However, recent studies proposed that humans may perceive complex carbohydrates and that the sensitivity to simple carbohydrates is independent of that to complex carbohydrates. Variation in ability to detect and perceive complex carbohydrates may influence food consumption. The aim of this study was to investigate the associations between oral complex carbohydrate sensitivity, body composition, and dietary intake in adults. Participants’ \( n = 34; \) 16 males, age 26.2 ± 0.4 years (range, 24 - 30 years); 18 females, age 29.4 ± 2.1 years (range, 24 - 55 years)] oral sensitivity towards complex carbohydrates (maltodextrin, oligofructose) was assessed by measuring detection threshold and suprathreshold intensity perception (gLMS). Height, weight, and waist circumference were also measured, and participants also completed a 4-day diet diary (3 weekdays; 1 weekend day) and a Food Frequency Questionnaire. There was large inter-individual variation in detection and intensity measures for complex carbohydrates. Pearson’s correlation coefficient revealed significant correlations between measures of oral sensitivity to complex carbohydrates, waist measurements, and dietary intake (energy and starch intakes). Detection threshold and suprathreshold intensity measures for each complex carbohydrate were treated as a grouping variable (tertiles) with participants categorised into more sensitive/experienced high intensity (1/3), normal sensitive/experienced moderate intensity (2/3), and less sensitive/experienced low intensity (3/3). Being more sensitive/experienced high intensity was associated with greater energy and starch intakes and a bigger waist measurement (all \( P < 0.05 \)). Taken together, these results reveal a novel association between complex carbohydrate sensing and the consumption of complex carbohydrates. Whether or not oral sensitivity to complex carbohydrate influences waist circumference or \textit{vice versa} still remains to be investigated.
Abstract

Study 3(a): The Associations between Oral Complex Carbohydrate Sensitivity, BMI, Liking, and Consumption of Complex Carbohydrate Based Foods

In comparison to simple carbohydrates, complex carbohydrates have been assumed tasteless to the human palate. Yet, recent work in this area suggests that humans may perceive complex carbohydrates and that their sensitivity to simple carbohydrates (i.e. glucose, sucrose) is independent from tasting complex carbohydrates. The aim of this study was to confirm whether humans could sense complex carbohydrates from a range of concentration levels; and if their oral sensitivity to complex carbohydrate relates to their BMI, liking, and consumption of complex carbohydrate based foods using a large sample group of adults. Participants’ \( n = 99; 7 \text{ males, age } 22.7 \pm 0.8 \text{ years (range, 22-24 years); } 92 \text{ females, age } 23.7 \pm 0.5 \text{ years (range, 19-47 years)} \) oral sensitivity towards complex carbohydrate (maltodextrin) and sweet taste function (glucose) was assessed by measuring detection threshold and suprathreshold intensity (gLMS). Participants were asked to complete an online version of a Food Frequency Questionnaire and a Likes and Dislikes Questionnaire. BMI was calculated using height and weight measurements. Hedonic ratings for complex carbohydrate and sweet solutions, as well as for a range of complex carbohydrate and sweet prototypical foods, were also measured. Consistent with previous findings, there was large inter-individual variation in detection and intensity measures for maltodextrin and glucose. No significant differences were found between oral complex carbohydrate sensitivity, BMI, and frequency of consumption of complex carbohydrate based foods measured. Similarly, no differences were observed between liking of complex carbohydrates, BMI, and food intake. All in, these
results from a large sample group further support the proposition that complex carbohydrates are not invisible to the human palate, and can be sensed in the oral cavity even at low concentration levels.

**Study 3(b): The Associations between Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, Liking, and Consumption of Ad Libitum Sweet and Complex Carbohydrate Milkshakes**

Excess energy intake is recognised as a strong contributing factor to the global rise of being overweight and obese. Our aim was to, first, investigate the effects of simple carbohydrate (glucose) and complex carbohydrate (maltodextrin) based milkshakes on ad libitum food intake, and second, to investigate the influence of simple and complex carbohydrate liking on ad libitum consumption of carbohydrate. Participants’ [(n = 56): 5 males: age 22.6 ± 0.2 years (range 22.0 – 23.0 years), 51 females: age 23.0 ± 0.6 years (range 20.0 – 41.0 years)] sensitivity towards maltodextrin (complex carbohydrate) and glucose (sweet) was assessed by measuring detection threshold and perceived intensity (gLMS). A crossover design was used to assess consumption of two different iso-caloric preload milkshakes and ad libitum milkshakes – 1) glucose based milkshake, 2) maltodextrin based milkshake. Ad libitum intake (primary outcome) and eating rate, liking, hunger, fullness, and prospective consumption ratings were measured. Hedonic ratings for complex carbohydrate and sweet solutions, as well as for a range of complex carbohydrate and sweet prototypical foods were also measured. Participants who were more sensitive towards complex carbohydrate (maltodextrin DT) consumed significantly more maltodextrin milkshake in comparison to less sensitive participants (P < 0.01). For the glucose milkshake, participants who had higher liking for sweet solutions consumed significantly more
Abstract

glucose milkshake in comparison to participants with lower hedonic ratings ($P < 0.05$). The results provide support regarding the role of the oral system (may be taste) surrounding sensitivity to complex carbohydrate and the consumption of complex carbohydrate foods within a meal. Perhaps, an unconscious mechanism encouraging consumption of complex carbohydrate may be advantageous to provide quantitative energy content for physiological functioning within a meal.

The three studies conducted as part of this thesis contribute to the growing knowledge surrounding sweet taste function, and the existence of oral complex carbohydrate sensitivity. Data from this thesis has reported that caloric sweeteners and NNS access at least partially independent mechanisms with respect to detection threshold and recognition threshold measures. However, at the suprathreshold intensity level, strong correlations between caloric sweeteners and non-nutritive sweeteners through weak, moderate, and strong intensity indicate a commonality in sweet taste mechanism for the perceived intensity range. Collecting a range of psychophysical measures across multiple sweeteners within a single group of individuals allows direct comparisons that cannot be made across prior studies. Furthermore, novel data in this thesis supports the existence and functionality of oral complex carbohydrate sensitivity and *ad libitum* consumption of complex carbohydrate foods, dietary intake, and body composition, adding to the growing body of evidence of an area worthy of further research.
List of Tables

Table 2.1 Sweetener concentrations used for determination of detection and recognition thresholds in Study 1 and Study 2.

Table 2.2 Sweetener and complex carbohydrate concentrations used for determination of detection thresholds in Study 3.

Table 2.3 Concentrations (weak, medium, and strong intensity) of sweeteners used for determination of suprathreshold taste intensity in Study 1, Study 2, and Study 3.

Table 2.4 Complex carbohydrate concentrations used for determination of detection thresholds in Study 2.

Table 2.5 Concentrations (weak, medium, medium-strong, and strong intensity) of complex carbohydrates used for determination of suprathreshold intensity in Study 2.

Table 2.6 Stimulus concentrations used for prototypical threshold testing in Study 1 and Study 2.

Table 2.7 Concentrations (weak, medium, and strong intensity) of prototypical tastants used for determination of suprathreshold taste intensity in Study 1 and Study 2.

Table 2.8 Sweet and complex carbohydrate based foods used for hedonic ratings in Study 3.

Table 2.9 Nutrient composition of sweet and complex carbohydrate milkshakes containing different amounts of glucose and maltodextrin (Study 3(b)).
Table 3.1 Stimulus concentrations used for prototypical threshold testing.

Table 3.2 Sweetener concentrations used for determination of detection and recognition thresholds.

Table 3.3 Concentrations (weak, medium, and strong intensity) of prototypical tastants used for determination of suprathreshold taste intensity.

Table 3.4 Concentrations (weak, medium, and strong intensity) of sweeteners used for determination of suprathreshold taste intensity.

Table 3.5 Detection and recognition thresholds for sweeteners (% w/v), including mean, standard error (SEM), and range.

Table 3.6 Detection and recognition thresholds (% w/v) for prototypical tastants, including mean, standard error (SEM), and range.

Table 3.7 Suprathreshold intensity ratings for prototypical tastants on gLMS, given by mean and standard error (SEM).

Table 4.1 Concentrations (weak, medium, and strong intensity) of tastants used for determination of taste intensity perception for prototypical tastants.

Table 4.2 Baseline characteristics of study participants (Mean values and standard errors).

Table 4.3 Taste thresholds (% w/v) of sweet tastants presented as mean, standard error, and range.

Table 4.4 Geometric mean (gLMS), standard error, and range sweetness intensity ratings of sweet tastants.
Table 4.5 Mean energy intake and macronutrient intakes (in percentages of energy intake) presented as mean and standard error.

Table 4.6 Consumption of added-sugar and specific sugar-sweetened foods in grams.

Table 5.1 Complex carbohydrate and sweetener concentrations used for determination of detection thresholds.

Table 5.2 Stimulus concentrations used for prototypical threshold testing (% w/v).

Table 5.3 Common sugars composition of the maltodextrin and oligofructose used in the present study.

Table 5.4 Concentrations (weak, medium, and strong intensity) of prototypical tastants and sweeteners used for determination of suprathreshold taste intensity.

Table 5.5 Concentrations (weak, medium, and strong intensity) of complex carbohydrates used for determination of suprathreshold intensity.

Table 5.6 Detection thresholds for complex carbohydrates and sweeteners (% w/v), including mean, standard error of mean (SEM), and range.

Table 5.7 Detection thresholds for four prototypical tastants (% w/v), including mean, standard error of mean (SEM), and range.

Table 5.8 Suprathreshold intensity ratings for four prototypical tastants on gLMS, given by mean and standard error of mean (SEM).

Table 6.1 Baseline characteristics of study participants (Mean values and standard errors).
Table 6.2 Detection thresholds (% w/v) and mean intensity ratings (gLMS) for complex carbohydrates presented as mean, standard error of mean, and range.

Table 6.3 Mean energy intake and macronutrient intakes (in percentages of energy intake) presented as mean and standard error.

Table 7.1 Sweetener and complex carbohydrate concentrations used for determination of detection thresholds.

Table 7.2 Common sugars composition of the maltodextrin used in the present study.

Table 7.3 Concentrations (weak, medium, and strong intensity) of glucose and maltodextrin used for determination of suprathreshold intensity.

Table 7.4 Sweet and complex carbohydrate based foods used for hedonic ratings.

Table 7.5 Baseline characteristics of study participants (Mean values and standard errors).

Table 7.6 Detection thresholds (% w/v) and mean intensity ratings (gLMS) for glucose and maltodextrin presented as mean, standard error of mean, and range.

Table 7.7 Sweet taste function (tertile groups) and frequency of reported sweet foods consumption.

Table 7.8 Oral complex carbohydrate sensitivity (tertile groups) and frequency of reported complex carbohydrate based foods consumption.

Table 7.9 Hedonic ratings (tertile groups) and frequency of reported sweet foods consumption.
Table 7.10 Hedonic ratings (tertile groups) and frequency of reported complex carbohydrate based foods consumption.

Table 8.1 Sweetener and complex carbohydrate concentrations used for determination of detection thresholds.

Table 8.2 Nutrient composition of sweet and complex carbohydrate milkshakes containing different amount of glucose and maltodextrin.

Table 8.3 Common sugars composition of the maltodextrin used in the present study.

Table 8.4 Concentrations (weak, medium, and strong intensity) of glucose and maltodextrin used for determination of suprathreshold intensity.

Table 8.5 Sweet and complex carbohydrate based foods used for hedonic ratings.

Table 8.6 Hedonic ratings and appetite ratings of fifty-six (n = 56) adults who consumed two types of milkshakes containing different amounts of glucose (sweet milkshake) and maltodextrin (complex carbohydrate milkshake) on two separate days.

Table 8.7 Drinking rates and meal durations of fifty-six adults (n=56) for ad libitum consumption of two types of milkshakes containing different amounts of glucose (sweet milkshake) and maltodextrin (complex carbohydrate milkshake).

Table 8.8 Detection threshold (% w/v) and mean intensity rating (gLMS) for glucose and maltodextrin presented as mean, standard error of mean, and range of fifty-six adults (n = 56).

Table 8.9 Hedonic ratings for sweet and complex carbohydrate solutions and prototypical foods presented as mean, standard error of mean, and range of fifty-six adults (n = 56).
List of Figures

**Figure 1.1** Graphic representation of the relationship between detection threshold, recognition threshold, suprathreshold intensity perception and chemical concentration levels.

**Figure 1.2** Schematic representation of lingual sweet taste receptor cells (TRC) and GI sweet TRC consisting of TIR2-T1R3 dimers.

**Figure 2.1** Flow diagram of the ascending forced choice triangle methodology.

**Figure 3.1** Frequency distributions of detection and recognition thresholds for: a) glucose, b) fructose, c) sucrose, d) sucralse, e) Rebaudioside A, and f) erythritol.

**Figure 3.2** Scatter plot matrix and spearman rank correlations of detection thresholds for sweeteners evaluated.

**Figure 3.3** Scatter plot matrix and spearman rank correlations of recognition thresholds for sweeteners evaluated.

**Figure 3.4** Mean psychophysical functions for suprathreshold taste intensity together with examples of a participant who experienced high intensity and low intensity: a) glucose, b) fructose, c) sucrose, d) sucralse, e) Rebaudioside A and f) erythritol.

**Figure 3.5** Scatter plot matrix and spearman rank correlations of sweetness intensity ratings for sweeteners evaluated.

**Figure 5.1** Frequency distributions of detection thresholds for: a) maltodextrin, b) oligofructose, c) glucose, d) fructose, e) sucrose, f) sucralse, g) Rebaudioside A, h) erythritol.

**Figure 5.2** (1) Spearman rank correlations between detection thresholds of maltodextrin and oligofructose. (2a-d) Correlations between detection thresholds of maltodextrin and caloric sweeteners: (2a) glucose; (2b) fructose; (2c) sucrose; (2d) erythritol. (2e-h) Correlations between detection thresholds of oligofructose and caloric sweeteners: (2e) glucose; (2f) fructose; (2g) sucrose; (2h) erythritol. (3a, 3b)
Correlations between detection thresholds of maltodextrin and non-nutritive sweeteners: (3a) sucralose; (3b) Rebaudioside A. (3c, 3d) Correlations between detection thresholds of oligofructose and non-nutritive sweeteners: (3c) sucralose; (3d) Rebaudioside A.

**Figure 5.3** Psychophysical curves of the group mean and examples of a participant who experienced high intensity and low intensity for (a) Maltodextrin (b) Oligofructose (c) Glucose (d) Fructose (e) Sucrose (f) Sucralose (g) Rebaudioside A (h) Erythritol. Included in each graph is the mean psychophysical curve as well as an example of a participant who experienced high intensity (highest curve) and a participant who experienced low intensity (lowest curve) for that complex carbohydrate/sweetener.

**Figure 5.4** (1) Spearman rank correlations of intensity ratings between maltodextrin and oligofructose. (2a-d) Correlations between intensity ratings of maltodextrin and caloric sweeteners: (2a) glucose; (2b) fructose; (2c) sucrose; (2d) erythritol. (2e-h) Correlations between intensity ratings of oligofructose and caloric sweeteners: (2e) glucose; (2f) fructose; (2g) sucrose; (2h) erythritol. (3a, 3b) Correlations between intensity ratings of maltodextrin and non-nutritive sweeteners: (3a) sucralose; (3b) Rebaudioside A. (3c, 3d) Correlations between intensity ratings of oligofructose and non-nutritive sweeteners: (3c) sucralose; (3d) Rebaudioside A.

**Figure 6.1** (A-B) BMI mean and standard errors between more sensitive/less sensitive participants and those who experienced high intensity/low intensity. (C-D) Waist circumference mean and standard errors between more sensitive/less sensitive participants and those who experienced high intensity/low intensity.

**Figure 6.2** (A-B) Mean energy intake per day and standard errors between more sensitive/less sensitive participants and those who experienced high intensity/low intensity. (C-D) Mean percentage energy from starch consumed per day and standard errors between more sensitive/less sensitive participants and those who experienced high intensity and low intensity.

**Figure 7.1** Number of participants who were recruited, screened, and completed both sessions.

**Figure 7.2** The study outline. The left chart represents the session outline for session one, the middle chart represents the session outline for session two, and the right chart represents the online questionnaires. Each session lasted about two hours. As the data collection was part of a laboratory class, participants were given intermittent breaks (teaching) in between each task lasting from 15-30 minutes.
Figure 7.3 Frequency distribution of detection thresholds for: a) glucose, b) maltodextrin.

Figure 7.4 Psychophysical curves of the group mean and examples of a participant experiencing high intensity and a participant experiencing low intensity for (a) Glucose (b) Maltodextrin. Included in each graph is the mean psychophysical curve, as well as an example of a participant experiencing higher intensity (highest curve) and a participant experiencing lower intensity (lowest curve) for glucose and maltodextrin.

Figure 7.5 (a) Spearman rank correlations between detection thresholds of glucose and maltodextrin. (b) Spearman rank correlations between intensity ratings of glucose and maltodextrin. The solid line in each graph represents the regression line.

Figure 7.6 Mean hedonic ratings for a range of (a) sweet solutions, (b) complex carbohydrate solutions, (c) sweet prototypical foods, and (d) complex carbohydrate prototypical foods.

Figure 7.7 (A-B) BMI mean and standard errors between more sensitive and less sensitive participants and those who experienced high intensity and low intensity according to sweet taste function and oral complex carbohydrate sensitivity measures. (C-D) BMI mean and standard errors between participants with high hedonic ratings and low hedonic ratings for both sweet and complex carbohydrate solutions and prototypical foods.

Figure 8.1 Number of participants who were recruited, screened, and completed both sessions. The dietary restraint score was measured according to factor one of the Three-Factor Eating Questionnaire. (1) Restrained eaters were defined as participants with a score on factor one of > 11 on the Three-Factor Eating Questionnaire.

Figure 8.2 The study outline. The left chart represents the session outline for session one, middle chart represents the session outline for session two, and the right chart represents the online questionnaires. Each session lasted about two hours. As the data collection was part of a laboratory class, participants were given intermittent breaks (teaching) lasting 15-30 minutes between each task.

Figure 8.3 Mean ± SEM *ad libitum* milkshake intakes by weight (g) (A) and energy (kJ) (B) of fifty-six adults (*n* = 56) who consumed sweet and complex carbohydrate milkshakes in random order.
Figure 8.4 Mean ± SEM hedonic ratings for preload and *ad libitum* sweet and carbohydrate milkshakes \((n = 56)\). The y-axis is the adjusted hedonic ratings from a nine-point hedonic scale. The x-axis represents the preload and *ad libitum* milkshakes measured.

Figure 8.5 Frequency distributions of detection thresholds \((n = 56)\) for: a) glucose, b) maltodextrin

Figure 8.6 Psychophysical curves of the group mean \((n = 56)\) and examples of a participant experiencing high intensity and a participant experiencing low intensity for (a) Glucose (b) Maltodextrin. Included in each graph is an example of the mean psychophysical curve, a participant experiencing high intensity (highest curve), and a participant experiencing low intensity (lowest curve) for glucose and maltodextrin. The y-axis is a numerical measure of intensity from the gLMS. The x-axis is the actual concentration in weight over volume percentage.

Figure 8.7 (A-B) *Ad libitum* milkshake intake mean and standard errors between more sensitive and less sensitive participants or those who experienced high and low intensity ratings (C-D) *Ad libitum* milkshake intake mean and standard errors between participants with high hedonic ratings and low hedonic ratings for both sweet and complex carbohydrate solutions and prototypical foods. For sweet taste function and sweet hedonic ratings, comparisons were only made for sweet milkshakes, and *vice versa* for complex carbohydrate.

Figure 8.8 *Ad libitum* milkshake intake mean and standard errors for participants with high hedonic ratings and low hedonic ratings for both sweet and complex carbohydrate milkshakes. For sweet hedonic ratings, comparisons were only made for sweet milkshakes, and *vice versa* for complex carbohydrate.
List of Appendices

Appendix A Screening Form (Study 1 and Study 2)

Appendix B Recruitment Flyer Study 1

Appendix C Recruitment Flyer Study 2

Appendix D Demographic Questionnaire – Online Survey and Compusense Script (Study 3)

Appendix E Ascending Forced Choice Triangle Form (Study 1 and Study 2)

Appendix F Detection Threshold Questionnaire – Compusense Script (Study 3)

Appendix G General Labeled Magnitude Scale (gLMS)

Appendix H Training Script gLMS

Appendix I Weight Bottles and Form – gLMS Standardisation

Appendix J Determination of Common Sugars in Foods by HPLC (Methods by the Australian Government National Measurement Institute)

Appendix K Examples of Five Prototypical Taste Detection and Recognition Threshold Questionnaire – Compusense Script (Study 1 and Study 2)
Appendix L Pictures of Prototypical Sweet and Complex Carbohydrate Based Foods Used to Measure Hedonic Ratings (Study 3)

Appendix M 9-point Hedonic Scale – Questionnaire and Compusense Script for Solutions and Prototypical Foods (Study 3)

Appendix N Likes Dislikes Questionnaire – Example of Printed Version (Study 3)

Appendix O Cancer Council Victoria Food Frequency Questionnaire – The Dietary Questionnaire for Epidemiology Study Version 2 (Study 1 and Study 2)

Appendix P Deakin Food Frequency Questionnaire – Printed Version (Study 3)

Appendix Q List of Collapsed Consumption Variables (Study 3a)

Appendix R Diet Diary Form (Study 2b)

Appendix S Non-Nutritive Sweetener Questionnaire (Study 1 and Study 2)

Appendix T Three-Factor Eating Questionnaire – Printed Version (Study 3b)

Appendix U Milkshake Hedonic and Appetite Ratings – Printed Version (Study 3b)
List of Abbreviations

BMI: Body Mass Index

DE: Dextrose Equivalent

DQESV2: The Dietary Questionnaire for Epidemiology Studies Version 2

DT: Detection Threshold

FFQ: Food Frequency Questionnaire

fMRI: Functional Magnetic Resonance Imaging

GI: Gastrointestinal

GIT: Gastrointestinal Tract

GIP: Glucose-dependent Insulinotropic Peptide

gLMS: General Labeled Magnitude Scale

GLUT2: Glucose Transporter 2

GLP-1: Glucagon-like Peptide 1

GPCR: G-protein Coupled Receptors
HPLC: High Performance Liquid Chromatography

ISO: International Standards Organisation

MSG: Monosodium Glutamate

NNS: Non-nutritive Sweetener

PLC-β2: Phospholipase C-β2

PYY: Peptide Tyrosine Tyrosine

RT: Recognition Threshold

SGLT-1: Sodium-glucose Transport Protein-1

ST: Suprathreshold Intensity Perception

TRC: Taste Receptor Cells

VFTD: Venus Flytrap Domain

WHO: World Health Organisation
Chapter One: Literature Review

1An abridged version has been published in Nutrients 2014, 6, 3431-3450; doi:10.3390/nu6093431, ‘The Role of Sweet Taste in Satiation and Satiety’

1.1 Introduction

Foods high in dietary carbohydrates in the form of simple carbohydrates and complex carbohydrates represent two essential sources of energy in our diet. Apart from certain fruits, there is much less sugar in plant food sources (e.g. tubers, legumes, grains) in comparison to complex carbohydrates, but it is sugars with their strong palatable sweetness that are the most taste-visible carbohydrates. (2) Driven by the palatability of simple carbohydrates, humans have over the years improved the refinement of pure sugar - started from the influx of sugar canes in India from its indigenous home, to the cultivation of sugar crystals from beets, and more recently, from corn. (3) Table sugar was on one occasion considered a luxury item for the upper class of society, but it is now a staple of modern life. (3-5)

Compared to simple carbohydrates, complex carbohydrates have long been presumed invisible to taste. (6, 7) However, there is increasing support from animal studies in the past decades indicating that rodents and even some non-human primates are attracted to the taste of complex carbohydrates derived from maltodextrin. (2, 8-17) It has also been reported that humans may perceive complex carbohydrates (maltodextrin, glucose oligomers), and that the sensitivity to simple carbohydrates (glucose, sucrose) was quantitatively different from complex carbohydrates. (18-21) Remarkably, although Lapis et al. (18) observed large individual variances between participants in terms of the α-amylase activity, taste responsiveness to maltodextrin
(Dextrose Equivalent (DE) 20, 10, and 5) was not significantly different between participants with high \( \alpha \)-amylase activity and those with low \( \alpha \)-amylase activity. While the human taste perception of complex carbohydrate (starch) hydrolysis products has been well investigated by Lapis et al. (18, 21), it needs replication and also extension. For example, the psychophysics of oral complex carbohydrate sensitivity and its associations with sweet taste function across multiple sweeteners has not yet been explored completely. It is important to note, that research that includes taste as a variable generally uses only one measure of taste function or a limited number of concentrations to measure oral sensitivity to complex carbohydrates. Furthermore, it remains to be verified, whether complex carbohydrates have a unique quality that does not overlap with the five basic taste primaries (sweet, sour, salty, bitter, and umami), and also if an individual’s sensitivity to complex carbohydrates remains stable over numerous testing sessions.

The present chapter will be divided into two main sections: (Section 1.2) on the role of sweet taste in satiation and satiety, (22) and (Section 1.3) on the evidence supporting the existence of oral complex carbohydrate sensitivity. Throughout this thesis, the terminology “oral complex carbohydrate sensitivity” will be used to refer to all types of complex carbohydrates and their derivatives including fibres (e.g. oligofructose), while not diminishing the prospect that oral perception of complex carbohydrate could be due to textural differences. Although the terminology “polysaccharide taste” has been recommended by Sclafani (2) to denote starch-derived saccharides containing three or more glucose units, it can be confusing as the word “polysaccharide” is generally used to describe complex carbohydrates, comprising more than ten monosaccharide units organised in chains. The word “oligosaccharide taste” (two to nine monosaccharide units) would be the more appropriate terminology,
nonetheless, it is not user friendly and it is unknown if perception of oligosaccharides is independent of textural differences. Therefore, at this stage of knowledge we recommend the use of “oral sensitivity to complex carbohydrate”, which correctly comprises all types of complex carbohydrates and their derivatives including fibres (e.g. oligofructose). Although dietary “carbohydrate” is an umbrella term for the monosaccharide and disaccharide sugars as well as starches and fibres, the term “sweet taste” has been collectively used to indicate sweetness. Thus “oral sensitivity to complex carbohydrate” would at the current state of knowledge be as correct as possible without oversimplifying tasting complex carbohydrates, but not easily confused with other sensations such as sweetness.

1.2 The Role of Sweet Taste in Satiation and Satiety

1.2.1 Overweight/Obesity

Obesity represents one of the largest preventable diseases worldwide and is thought to be a key contributor to a number of major health problems, such as high blood pressure, stroke, type 2 diabetes, coronary heart disease and some forms of cancer. (23-27) According to the Landmark Global Burden of Disease report, obesity was emphasised as the prominent cause of disability worldwide and as a more significant health crisis worldwide than starvation and/or malnutrition. (28) Throughout the past 30 years, obesity has been a prominent problem and has increased globally in all classes of socioeconomic status in both developing and developed countries. (29) In the year 2014, the global estimated total number of overweight adults aged 18 years and above was 1.9 billion. (23) Among these, a staggering estimated number of over 600 million adults were obese. (23) If current trends remain, by the
year 2030, it is projected that 2.16 billion adults would be overweight and 1.12 billion adults would be obese, increasing the burden of obesity-related morbidity and mortality. (30, 31)

Increased energy intake, in particular greater intakes of sweet food, is thought to be one of the major contributors to the global rise in being overweight and obese. (31, 32) Foods that taste sweet have long been associated with dietary energy. (32, 33) As an example, in the current day, excessive consumption of sugar, particularly in sugar-sweetened beverages, has been linked to the rising rates of obesity worldwide. (34-42) Refined sugars added to food and beverages have little to no nutritional value and contribute to increased energy intake. (43, 44) However, at the same time, there are published critical reviews, systematic reviews, and meta-analysis of randomised controlled experiments that have found no conclusive evidence that sugar-sweetened beverages consumption has uniquely contributed to being overweight or obese. (45, 46) One potential way to tackle the current obesity crisis is to reduce energy intake through using non-nutritive sweeteners (NNS). (47, 48) Replacing sugar with NNS in a beverage or food decouples sweetness from energy value while maintaining sweetness. (48-50) Unfortunately, the scientific literature on the topic of sweetness and energy intake is divided. (32, 50) NNS, near zero-energy sugar replacements, were introduced before World War I; (51) however, it was not until the 1990s that NNS have been widely promoted as “healthier alternatives” in commercialized goods, such as low-energy soft drinks, processed foods and confectionaries. Yet, the prevalence of obesity has continued to increase.

The continued increase in the prevalence of obesity worldwide despite a logical solution to decrease energy intake suggests two likelihoods. One, NNS did effectively reduce energy intake from sweet foods, and increases in obesity are due to
other factors, such as the increased consumption of cheap, energy-dense foods. (31) Two, NNS did not reduce energy intake. (52) Sweetness without the associated energy may actually increase appetite and encourage consumption of other foods. (50) If the second possibility holds true, then perhaps what promotes weight gain is more complicated than decoupling taste and energy, with physiological systems demanding, via appetite, the “sweet” promise of energy being delivered. The aim of this review (Section 1.2) is to discuss possible associations between sweet taste function (tasting caloric sweeteners and NNS) with satiation and satiety. The possible associations between sweet taste mechanisms within the oral cavity, GI tract (GIT) and the brain systems towards both caloric sweeteners and NNS, and sweet taste function will also be reviewed.

### 1.2.2 Taste and Its Function

The sense of taste is one of the traditional five senses (sight, hearing, taste, smell and touch) and refers to the sensation derived when non-volatile chemical molecules stimulate receptors sited on taste cells in the surface areas of the tongue, soft palate and the oropharyngeal region. (53) Taste is stimulated through the activation of taste receptor cells (TRC) found in the surface regions throughout the oral cavity. (53) Once the TRC are activated, electrical impulses are transmitted via the sensory afferent fibres to the brain areas involved in the corticol processing of taste, and a taste perception associated with the chemical will be experienced. (54) TRCs are housed within the taste buds, which are distributed across three different types of tongue papillae (i.e. circumvallate, foliate and fungiform papillae). (53)
The prevailing understanding at present is that the human taste system is now widely accepted to include five basic tastes (sweet, sour, bitter, salty, and umami taste), and fat taste being accepted by a few. (55-65) From an evolutionary perspective, it is postulated that the human taste system functions as a gatekeeper of the digestive system to ensure that we consume essential nutrients for survival and functioning, while rejecting potentially harmful or toxic foods. (66) For example, a salty taste quality signals the presence of either sodium or minerals; umami indicates the presence of proteins; excessive sour taste signals spoiled food; bitter taste quality often indicates the presence of poisons; and sweet taste indicates the presence of energy in the food. (58, 66) It has been suggested by Beauchamp (67) recently that sweet taste perception or the degree of sweetness in general is not a good proxy for the amount of energy available in a food. Rather, a detection mechanism encouraging consumption for complex carbohydrate independent from sweet taste may be advantageous to provide quantitative information about the energy (glucose) content for physiological functioning. (67, 68) Thus, it could be argued that the physiological regulation and functional significance of sensing low amounts of complex carbohydrates could be an advantage, as complex carbohydrates represent a major source of energy for physiological functions.

A taste perception for a particular taste quality is experienced when the concentration of that particular solute in the oral cavity reaches a level that activates a taste receptor. (54) For instance, when 1 mM sucrose is dissolved in water, an individual may find it challenging to differentiate the sucrose-containing solution from plain water. However, as the concentration of sucrose is increased, differentiation becomes possible. (54, 69, 70) The lowest concentration level at which a difference can be detected is termed the sucrose detection threshold (DT) (Figure 1.1). At this
concentration level, the individual cannot accurately identify the sucrose solution as sweet, and only when the concentration of sucrose is further increased does the sweet taste quality become apparent. (71) The lowest concentration at which this occurs is termed the sucrose recognition threshold (RT). (54, 71, 72) As sucrose is progressively added beyond this point, the perceived sweetness will range from just perceivable to strong, until it reaches the individual’s terminal threshold for sucrose, beyond which any increase in concentration no longer causes consequential increase in perceived sweetness intensity. (54, 73, 74) Perceived sweetness above the RT is defined as the suprathreshold intensity perception (ST) range. (69, 74)

Theoretically, it seems reasonable to expect that an individual’s sweetness DT, RT, and ST might be interrelated. (54, 69, 74-76) An example of this hypothetical model was observed from a bitter compound, 6-n-Propylthiouracil (PROP). For example, an individual who is able to detect and/or recognise bitterness from PROP at a lower concentration level may, when tasting a concentrated PROP solution, be more likely to experience greater bitterness intensity than another individual with a higher bitterness DT for PROP (i.e. strong negative correlation between DT and ST for PROP). (54, 77) This, however, has not been confirmed for sweet compounds. (70, 78)
Figure 1.1 Graphic representation of the relationship between detection threshold, recognition threshold, suprathreshold intensity perception and chemical concentration levels. The x-axis of the graph represents the chemical concentration level from no concentration of sucrose in the aqueous solution (0 molar) to a saturated sucrose solution. The y-axis represents the general Labeled Magnitude Scale (gLMS) from no perception to a conjectural terminal threshold. (I) Small amount of sucrose diluted in an aqueous solution could not be detected at a low concentration. (II) A detection threshold is reached whereby the sucrose solution can be told apart from water. At this stage, the taste quality remains unidentified. (III) The recognition threshold is reached whereby the correct taste quality can be recognised. (IV) Suprathreshold intensity perception is defined as the dynamic phase where the perceived intensity of sweetness jointly increases to a hypothetical asymptote as the concentration of sucrose increases. Further increases following the dynamic phase no longer cause subsequent increases in perceived intensity.
1.2.3 Peripheral and Central Mechanisms for Sweet Taste Detection

Taste processing involves a multifaceted flow of events involving taste, learning, memory and the reward systems. (47) It is thought that the individual’s ability to detect or sense sweetness in the oral cavity (initial process of sweet taste perception) is one of myriad factors influencing food acceptance, and as such, taste may play a role in modulating food acceptance and/or energy intake. (33) In addition to an individual’s ability to detect sweet taste within the oral cavity, emerging evidence now suggests that the sweet taste signalling mechanisms identified in the oral cavity also operate in the gastrointestinal (GI) system, which may possibly influence satiety. (79-87) Therefore, understanding the individual differences in detecting sweetness in both the oral and GI system towards both caloric sweeteners and NNS and the functional role of the sweet taste system may possibly be an important factor in understanding the causes for excess energy intake. In this review, evidence of possible associations between sweet taste mechanisms within the oral cavity, GIT and the brain systems towards both caloric sweeteners and NNS, and sweet taste function are summarised and discussed. The possible links between sweet taste function, satiation, and satiety towards both caloric sweeteners and NNS are also discussed. The term “satiation” in this review refers to the process that contributes to the cessation of a meal, whereas “satiety” is mainly associated with the post-absorptive effects of consuming foods and, therefore, will be considered as the time intermission until the next eating episode. (49)
1.2.3.1 Peripheral Mechanisms for Sweet Taste Detection

It has been shown that there are similarities for sweet detection in the oral cavity and GIT, between oral sweet TRC and the GI sweet TRC (Figure 1.2). (82, 88-93) The existence of an almost identical nutrient-sensing mechanism in the oral cavity and GIT seems reasonable, given that both are part of the alimentary canal and responsible for the identification of nutrients and non-nutrients in foods. (94) Further, both the oral cavity and GIT initiate the appropriate functional responses, such as taste perception (oral cavity) and hormone release (e.g. satiety hormones) (GIT). (94)

Figure 1.2 Schematic representation of lingual sweet taste receptor cells (TRC) and GI sweet TRC consisting of TIR2-T1R3 dimers. Once the sweet substances bind to the sweet taste receptors, intracellular signalling elements are activated, including α-gustducin, which, in turn, activates phospholipase C-β2 (PLC-β2). The stimulation of PLC-β2 leads to the generation of IP3, where the IP3R3 further activates the calcium ions from the endoplasmic reticulum. After the calcium ions are released, the TRPM5 channel is activated, resulting in sodium entry in the plasma membrane. Sodium entry leads to depolarisation, thus inducing calcium entry through the calcium channel. The calcium ions then induce the discharge of neurotransmitters from oral sweet TRC, which are then relayed via the afferent nerve to the brain areas involved in sweet taste processing. In the GI sweet TRC, satiety hormones, such as peptide tyrosine tyrosine (PYY), glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), are released upon secretion of calcium ions within the cell. These satiety signals are then relayed to the brain via the vagal nerve.
1.2.3.2 Sweet Taste Detection in the Oral Cavity

There is strong evidence in the literature that the receptor on the sweet TRC is a heterodimer of two G-protein coupled receptors (GPCR), T1R2 and T1R3. (53, 94-106) For NNS, a single knockout of either T1R2 or T1R3 eliminates all behavioural preference for NNS. (107, 108) The T1Rs are class C GPCRs, which entails a large extracellular region (N termini) that binds ligands and form structures that resemble a “Venus flytrap domain” (VFTD), (98, 100, 109, 110) which is connected to the transmembrane via a cysteine-rich domain. (111) It has been suggested that the VFTD of T1R2 targets a large variety of sweet substances (natural sweeteners and most of the NNS); whereas the VFTD of T1R3 targets other NNS, such as cyclamate, and the sweet receptor blocker lactisole; and the cysteine-rich domains activate sweet proteins. (95, 111-115) This evidence raises the possibility that differences in sweet perception of different sweeteners may be due to differences in downstream signalling pathways, or even differences in receptor kinetics as a result of binding to different sites of the sweet taste receptor. (106, 111, 116)

Once the sweet compounds bind to the sweet TRC, intracellular signalling elements are activated, including α-gustducin, which, in turn, activates phospholipase C-β2 (PLC-β2) (Figure 2.2). (117, 118) The stimulation of PLC-β2 leads to the generation of diacyl-glycerol and inositol-trisphosphate (IP3), where isoform 3 of the IP3 receptor (IP3R3) further mobilises the calcium ions from the endoplasmic reticulum into the cytoplasm. (119) Once the calcium ions are released from the endoplasmic reticulum, the TRPM5 channels are activated (i.e. calcium-activated cation channel resulting in sodium entry) in the plasma membrane of the sweet TRC. (120) Sodium entry resulting from the activation of the TRPM5 leads
to depolarisation in the plasma membrane. (99, 121) Consequently, this induces calcium entry through the voltage-gated calcium channel. (98, 120) Once activated by sugar or certain NNS, sweet TRC transmit the information via sensory afferent fibres to the brain areas involved in sweet taste processing. (89) The oral sweet information is also sent to the stomach via the vagus nerve to proceed with the cephalic phase response (i.e. gastric juice secretion). (89, 121) This process further initiates functional responses in the GIT, such as glucose uptake and hormone release. (121) Support for this model comes from the evidence that deactivating mutations of PLC-β2, TRPM5, or IP3R3 severely weaken behavioural responses to sweet. (122, 123)

1.2.3.3 Sweet Taste Detection in the Gastrointestinal Tract

It is now evident that the expression of the functional sweet TRC is not restricted to the oral cavity, but is also present in other parts of the GIT, including the intestinal enteroendocrine cells (82, 91) and other tissues, such as the pancreas. (124) However, individuals can only consciously perceive sweetness upon the activation of the sweet TRC in the oral cavity, because sweet TRC in the GIT and other tissues do not convey taste perception. (125) Likewise, with the oral sweet TRC, the GI sweet TRC also expresses α-gustducin, PLC-β2 and TRPM5 in the GIT (Figure 1.2). (126) Upon stimulation with sugars or NNS by the GI sweet TRC, digestive and absorptive processing of the ingested food is further coordinated by the secretion of gut hormones, including PYY, GLP-1 and GIP, further regulating insulin release from pancreatic β-cells. (79, 85, 126) These gut hormones are associated with the metabolism of nutrients and fullness. (126) In
addition to the regulation of incretins, the GI sweet TRC also controls for glucose uptake in the intestinal epithelium. (127) Two types of glucose transporters facilitate the glucose uptake in the GI lumen: sodium-glucose transport protein-1 (SGLT-1) and glucose transporter 2 (GLUT2). (80, 96, 127)

1.2.3.4 Non-Nutritive Sweeteners and Their Role in the Gastrointestinal Tract

As previously described, the sweet TRC can bind to sweet molecules of varying structures, including caloric sugars and a range of NNS. However, the metabolic fate of NNS varies in the GIT depending on the chemical structure of each NNS. (125) It was suggested that NNS, such as sucralose is partially digested or metabolised in the body, whereas aspartame breaks down into smaller compounds to be digested in the intestine. (128) Given the chemical heterogeneity of these NNS, it is surprising that these sweeteners still have the ability to activate sweet TRC in the oral cavity, generating similar signals to caloric sugars, resulting in similar sweetness signals in the brain. (98, 125, 126, 128) However, the signalling transduction and downstream actions, such as satiety hormone release in the GI system, upon activation by NNS is controversial. Some studies support the hypothesis that NNS bind to the sweet TRC on the enteroendocrine cells, resulting in similar signal transduction and downstream actions to caloric sugars, such as satiety hormone secretion in humans. (80, 125, 126, 128) Nevertheless, the majority of in vivo studies have failed to confirm this. (129) Fujita et al. (129) reported that despite oral taste receptor sensitivity to NNS, four different NNS (acesulfame K, Rebaudioside, sucralose and D-tryptophan) did not evoke GLP-1 or GIP release in
rats. It is noteworthy that in this study, the concentrations of sweetener given were 1000-fold in excess of the concentrations used in NNS-sweetened products, such as diet soda. (125) Similarly, in human studies, NNS, such as sucralose, aspartame and acesulfame K, did not evoke any GLP-1 release. (130-132) The inability of NNS to initiate any GLP-1 hormones questions previous notions where GI sweet TRC were thought to be involved directly in GI glucose uptake in the intestinal epithelium. (133)

Recent studies investigating the role of SGLT-1 in cultured L-cells have suggested that perhaps the SGLT-1 is more involved in sugar sensing in the GIT in comparison to the T1R2-T1R3 dimer. (133) SGLT-1 has an important function in glucose homeostasis, as it is the primary transporter of dietary sugars in the GI lumen (134) and has been found to be involved in the mechanism of glucose-induced incretin release in cultured mice L-cells. (135, 136) It is important to note that Moriya et al. (137) did not report a marked increase in GLP-1 upon administration of NNS, such as saccharin and sucralose, to non-metabolisable SGLT-1 substrates. As SGLT-1 functions as the primary gut glucose sensor instead of GI sweet TRC, collectively, this further suggests that perhaps NNS are able to generate taste signals from the sweet TRC in the oral cavity, but do not stimulate gut receptor mechanisms that are involved in satiety. A full description of the signalling pathways that facilitate sweet taste perception for both caloric sugars and NNS within the GIT in human studies warrants further investigation, as it is of major importance, due to their possible involvement with GI functions, including satiety hormone release and, thus, appetite and energy intake.
1.2.3.5 **Sweet Taste Processing in the Brain**

The previous subsections have focused on the sweet taste signalling pathways in the oral cavity and the GI system that potentially influence food preference and acceptance. The information generated from the oral cavity is forwarded to the brain via the sensory afferent fibres. (138-140) Taste information is then transmitted to the primary taste cortex. (141-143) The neurons in the primary taste cortex relay information to pathways involved in the central processing of the food reward along the dopaminergic midbrain. (141-143) Neurons within the dopaminergic midbrain subsequently inform other brain regions that are involved in the food reward system. (141-143) The brain regions known to date that are involved in the food reward system include the orbitofrontal cortex, caudate nucleus and amygdala. (143) The activation of the brain centres involved in the reward pathway consequently releases dopamine, a neurotransmitter frequently linked with reward. (143) Brain autonomic centres may also relay and receive information with the GI system via the vagal nerve to coordinate with satiety hormones to prepare the digestive system for the incoming carbohydrate-rich food. (143, 144) Although hypothetical at this stage, the body’s food reward system, along with the sugar sensing mechanisms in the oral cavity and GIT, may play a crucial role in the regulation of eating behaviour, as well as possibly controlling the amount of dietary energy an individual consumes. (145) The question now is whether NNS can impact the central taste and reward pathways similarly to caloric sugars in the brain, given that NNS are able to activate TRC in the oral cavity, or whether the brain itself is able to identify NNS and, thus, sends information to the gut system not to activate SGLT-1. That is, does one feel the same level of satisfaction and pleasure upon ingesting NNS-sweetened foods as compared to sugar or does appetite increase or satiety decrease upon ingestion of foods containing NNS?
1.2.3.6 Are Non-Nutritive Sweeteners Equally as Rewarding to the Brain as Caloric Sugars?

Recent literature using functional magnetic resonance imaging (fMRI) data investigating if NNS are equally as rewarding to the brain as caloric sugar revealed some interesting findings. In a study by Smeets et al. (146), 20 healthy men were asked to rate the satisfaction and sweetness of solutions sweetened with either sucrose or a NNS while undergoing fMRI scans. It was found that the human brain responds differentially to sucrose and NNS, particularly in the activation of striatum (i.e. striatum, a brain region involved in the reward pathway, was not activated upon ingestion of a NNS sweetened solution in comparison to sucrose). (146) Similarly, in another study by Frank et al. (147), researchers found that both caloric sugar and NNS were able to activate the primary taste pathway in the brain, but only caloric sugars were able to activate a significant response from brain regions involved in the brain’s reward pathway. These findings suggest that perhaps the brain’s reward pathway is adapted to favour caloric sweeteners in comparison to NNS. (145) However, an interesting question is whether there are differences between habitual consumers and non-habitual consumers of NNS. A study by Green and Murphy (148) found that diet soda drinkers demonstrated greater overall activation to sweet taste from both caloric sugar and NNS in several reward processing brain regions, compared to a non-diet soda drinker group. Within the diet soda drinkers, there was no difference in the brain’s response to both caloric sugar and NNS. (146, 148) Additionally, a study by Rudenga and Small (149) suggested that repeated consumption of NNS alters brain responses to caloric sweeteners. In this study, participants (n = 26) were asked to ingest sucrose solutions while participating in a fMRI scan. (149) It was observed that participants who reported higher intake of NNS showed a reduced amygdala response to sucrose.
suggesting that habitual use of NNS may be associated with brain changes that could influence eating behaviour. (149) The lack of response to sweet taste among habitual consumers of beverages sweetened with NNS can potentially be explained as a result of the repeated experience of sweetness without energy. It is also important to note that these studies do not convey causality.

1.2.4 Possible Role of Sweet Taste Function

The latest progress in our knowledge surrounding sweet taste detection mechanisms for caloric sugars and NNS within the mouth, the brain and the GIT, (56, 82, 88-100, 109, 110, 128, 138-145) combined with their fundamental role in regulating appetite and, thus, dietary energy intake, (125, 128, 133, 137, 143, 144, 150) suggest that abnormalities in any or several of these nutrient sensors may be an underlying factor of why some individuals consume more energy. (74, 151, 152) As such, variations in sweet taste detection (initial contact with food) towards caloric sugars and NNS and how these may influence appetite (satiation and satiety) are of high interest. Although generally hypothetical at the present time, the links between sweet taste function, satiation, satiety and weight suggest an intriguing model to understand being overweight and obese.

1.2.4.1 Individual Differences in Sweet Taste Function

As with all taste qualities, there is large individual variation in the capability to perceive sweet taste (sweet taste function). Inter-individual differences or variability in sweet taste function has been previously observed for sucrose, (70, 153-158) and a range of sweeteners. (159-161) These large individual variations in the ability to
perceive sweet taste may perhaps explain why some individuals, in particular those who are less sensitive or those who experienced low intensity to sweetness at a fixed concentration of sugar, consume more sugar and/or have greater dietary intake in comparison to those who are more sensitive or those who experienced high intensity to sweetness at the same concentration of sugar. (157) Individuals who are less sensitive or experienced low intensity to sweetness are postulated to be at risk of long-term health outcomes, such as obesity, as they will need to consume more sugar to have the same sweet taste sensation compared to those who are more sensitive or experienced high intensity. (157) For example, an individual who is more sensitive or experienced high intensity to sweetness may only need to add a teaspoon of sugar to their coffee in order to achieve the desired sweetness sensation. Conversely, a less sensitive individual or one who experienced low intensity may need to add more teaspoons of sugar in order to achieve a similar level of sweetness sensation, thus increasing total energy intake. Therefore, studying the individual variance in sweet taste function may be an important underlying factor to understand why some individuals may consume excess dietary sugar.

Variations in sweet taste function are likely to be influenced by environmental and genetic factors. A study investigating the associations between common variation in genes encoding the human sweet taste system and the individual variation in sweet taste function among 160 unrelated individuals found that the taste threshold and suprathreshold sensitivities of participants for sucrose were significantly associated with the genetic variation occurring in the GNAT3 gene (which encodes for α-gustducin). (162) Interestingly, the genetic variations of the GNAT3 gene also explained 13% of the variation in sweet taste perception. (162) Another study of female monozygotic and dizygotic twins reported that genetics accounts partly for the ability to perceive the intensity of a sweet (sucrose) solution and for the liking of sweet
foods, (163) suggesting that liking of sweet-tasting foods is a multifactorial, polygenic trait. (164) Other evidence from a genotyping study involving 1137 participants showed genetic variation in the T1R2 gene was associated with significant differences in total free sugar consumption among overweight and obese populations, but not those of healthy weight. (165) Nevertheless, as the study did not assess sweet taste function, it is impossible to understand if the measured genetic variation or the intake of free sugars was associated with perceptual measures of sweet taste. (166)

Recent studies have also suggested that the individual differences in detecting or recognising sweetness may perhaps be influenced by sensitivity to other taste qualities, such as bitter taste. (167-169) For example, Zhang et al. (169) found an inverse correlation in the relationship between the DT for sucrose and the fungiform papillae density (i.e. a higher density of fungiform papillae is associated with individuals that are more sensitive to sucrose). Given that the commonly used NNS, such as aspartame, acesulfame K, sucralose and Rebaudioside A, have been reported to have a long-lasting bitter after-taste, (170, 171) it is essential to investigate further if consumption of NNS is driven by an individual’s sensitivity to both sweet and bitter taste.

In addition to an individual’s genetic predisposition, sweet taste function may be influenced by environmental factors, such as dietary intake. One recent small randomised controlled trial looked at the effect of reducing intake of simple sugars on DT and suprathreshold intensity for sucrose. (116) In this study, 13 participants completed a three-month low-sugar diet and 16 participants (control group) remained on their normal diet. (116) No significant changes in sweet DT were found during the intervention in both groups, but the low-sugar diet group rated sweet foods as more intense in months two and three. (116) A similar, but weaker effect on rated sweetness
intensity for flavoured beverages was also found. (116) However, a one-month post-intervention follow up revealed that the sweetness intensity ratings for the low-sugar diet group had returned to their baseline measurements. (149) Nevertheless, whether or not habitual diet can alter sweet taste function or vice versa is still unclear. In a cross-sectional study ($n = 85$ adults) investigating the relationship between sweet intensity perception and dietary intake, no significant associations were found between perceived sweet intensity and dietary energy intake relating to sugar consumption. (158) However, in this study only a single measure of sweet taste was used, and perhaps, using another measure of sweet taste function (i.e. DT, RT, and suprathreshold intensity perception) could yield different outcomes. (158) Furthermore, it is also possible that the association between sweet taste and sugar intake is minimal due to the multiple other factors that may influence dietary intake, one of which will be binary interactions of dietary sugars with other components of food, such as dietary fat. (166) It is also important to acknowledge that there are large individual differences in preference and desire for sweetness (172, 173) and long-term exposure to sweet taste during infancy has also been shown to have an effect on young children’s preferences for sweet taste. (174, 175) Therefore, it is also reasonable if no associations were observed between sweet taste and dietary energy relating to sugar consumption, because some people may like increasing concentrations of sucrose, (176, 177) whereas some people may only like increasing concentrations of sucrose up to a bliss point (inverted U-shape) whereby liking decreases following this point with increasing concentration of sucrose. (178)
1.2.5 Implications of Decreased Sweet Taste Function, the Gastrointestinal System, and Satiety

1.2.5.1 Implications of Sweet Taste Function on Body Mass Index

The role of taste function in promoting intake of specific foods or ingredients associated with obesity has long been an area of interest, but with mixed experimental support. (55, 56, 63, 158, 179-185) In regards to sweet taste, whether or not body weight can alter sweet taste function or vice versa is still unclear. Some have reported an inverse association between BMI and sweet taste function (decrease in BMI were associated with increased sweet taste function). (74, 185-188) For example, a recent study of normal weight ($n = 52$) and overweight/obese ($n = 51$) participants found that those with higher BMI had significantly higher DT for sucrose (i.e. higher DT = differentiated the sucrose-containing solution from plain water at a higher concentration level). (188) Similarly, a study measuring sweetness intensity of candy rated on the general labeled magnitude scale (gLMS) using a large sample size ($n = 3740$), found a weak positive association between body size and sweetness intensity perception ($r = 0.04$). (74) On the other hand, a large body of evidence indicates that there is no significant association between BMI and sweet taste function. (158, 189-196) For example, a recent study assessing sweet taste DT using the ascending forced choice triangle methodology ($n = 149$; 41 overweight, 52 obese, and 56 normal weight) revealed no significant differences in sweet taste DTs between the weight status groups. (196) However, discrepancies between studies may be attributed to differences in the psychophysical techniques used to measure sweet taste function (74, 166) as research has shown that no single psychophysical measure reflects taste function in totality. (70) There are three perceptual dimensions of sweet taste function, namely detection threshold, recognition threshold, and suprathreshold intensity, each of which
is independent of the other. (54, 70) It is also probable that the inconsistencies between studies may be attributed to the type of sweetener used, that is, caloric sweeteners and NNS. In the past few decades, new NNS have been introduced into our diets. A confounding factor in this area is that NNS generally contain only negligible amounts of kilojoules thereby decoupling sweetness from energy value. (49) Questions remain regarding how these non-nutritive sweet compounds relate to caloric sweeteners (i.e. the perceptual relationships between caloric and NNS using a range of psychophysical measures within a single group of individuals), and the associations between sweet taste function, body composition, and dietary intake. Similar complexities were also found in studies investigating the link between sweetness liking and BMI, where most data showed no link between hedonics of sweetness and body size. (177, 190, 192, 194, 195, 197-199)

1.2.5.2 Sweet Taste Appetite

The influence of sweetness on appetite has been of interest over the past few decades. However, the specific role of sweet taste in appetite regulation is controversial (reviewed in Sorensen et al. (49) and Rolls (200)). For example, some studies, but not all, show that sweet taste in itself can stimulate hunger, (201-204) whereas some failed to show an effect of sweet taste on appetite and food intake in subsequent meals. (205-209) It is possible that some studies show no effect of sweet taste on appetite, as there may be individual differences in sweetness level related to appetite. (204) In a study by Tordoff and Alleva (204) the levels of sweetness of chewing gums were manipulated using different concentrations of aspartame (NNS). Interestingly, it was found that the most effective sweetness level to increase hunger differed among most individuals. (157) In two other studies investigating the
palatability of yogurts with different concentrations of aspartame (NNS) and sucrose, yogurt intake was found to be greater at the sweetness level most preferred by the individual. (210, 211) Using a 24-hour food diary, subsequent food intake (food intake in the 24-h after yogurt intake) was also reported to be significantly greater following consumption of the yogurt with the preferred sweetness level suggesting that the yogurt with the sweetness level most preferred by the individual stimulated appetite. (210, 211)

1.2.5.3 Links between Sweet Taste Perception of Caloric Sweeteners and Non-Nutritive Sweeteners and Satiation

The process of satiation or fullness is, to a large degree, mediated by sensory processes, generated from the flavour attributes of foods (sight, taste and smell). (212) When a food is eaten to satiety, the pleasantness of the flavour properties of that food decreases more than for other foods. (213) This is termed “sensory-specific satiety”. (214) Development of sensory-specific satiety is predominantly associated with sensory stimulus accompanying the ingestion of food, as opposed to the post-absorptive effects of consuming these foods. (165) A key factor that contributes to the cessation of the meal is thought to be the diminished pleasure from exposure to the flavour. (87)

The influence of sweet protein flavours on satiation has sparked some interest over the past few decades. However, how different caloric sweeteners and NNS influence satiation is not fully clarified. A study by Rolls et al. (215) investigated the effects of sucrose and aspartame-sweetened (NNS) gelatines on appetite ratings and overall food intake in a meal using a crossover design. Participants were instructed to consume either a high-energy gelatine (sweetened with sucrose) or a lower-energy
version (sweetened with aspartame). Interestingly, there were no significant differences found between rated sensory-specific satiety, hunger, fullness, and desire to eat following consumption of both versions of the gelatines. There were also no significant differences in the amount of high- and low- energy versions of gelatines consumed in a meal despite differences in energy intake. (215) Similarly, another study by Rolls et al. (208) further investigated if there were differences in terms of short-term appetite and food intake following consumption of drinks sweetened with either sucrose or aspartame (NNS) in men. The results suggest that there were no differences between consuming a sugar-sweetened beverage or an aspartame-sweetened (NNS) beverage on short-term hunger ratings or subsequent food intake. (208) Similar finding has been reported by Mattes (375) in a crossover trial where participants consumed equicaloric breakfasts including unsweetened or sweetened (sucrose or aspartame sweetened) cereal for five consecutive days, during which hunger and energy intake following consumption of cereals were recorded. It was found that the addition of caloric (sucrose) or NNS (aspartame) to a breakfast cereal had no significant effect on food intake at the next meal or over the day suggesting that NNS may not have paradoxical effects on stimulating appetite in comparison to sucrose. (375) However, the role of sweet taste (caloric sweetener versus NNS) in energy intake and appetite regulation is controversial. One recent crossover study by Tey et al. (216) investigated the effects of caloric (high-energy; sucrose) and NNS sweetened (low-energy; aspartame, Mogroside V, Rebaudioside A) preload beverages on subsequent energy intake. In this study, participants consumed one preload beverage an hour before ad libitum lunch. (216) After consumption of the NNS sweetened beverages, participants consumed significantly greater ad libitum lunch in comparison to lunch ingested after consumption of the caloric sweetened preload beverage. (216) Furthermore, participants were found to fully compensate energy by the end of the test day after
consumption of the NNS sweetened beverages (i.e. no significant difference in total
daily energy intake was found after consumption of caloric and NNS sweetened
preload beverages). (216)

Two other studies examining the effects of sweet taste on short-term hunger
were conducted among habitual high and low consumers of beverages sweetened with
NNS. (217, 218) It was found that the effects of sweet and non-sweet lunches on short-
term hunger differed significantly in terms of individuals’ habitual consumption of
sweet low-energy drinks (i.e. an increase in short-term appetite ratings in response to
sweet taste was demonstrated among low consumers of beverages sweetened with
NNS, whereas high consumers did not show this increase). (167, 168) Consistent with
what was previously mentioned, it is possible that the appetite ratings differed between
habitual consumers and non-consumers of NNS drinks because of other contributing
factors, such as adaptation (i.e. impaired sweet taste perception and sweet reward
pathways following repetitive consumption of a particular sweet stimulus), which may
influence food behaviours. Furthermore, the influence of knowledge or beliefs about
the energy content of foods may also lead to overconsumption of foods [see review by
Mattes & Popkin (50)].
1.2.5.4 Links between Attenuated Sweet Taste Perception, the Gastrointestinal System, and Satiety

As previously mentioned, peripheral mechanisms for identifying sugars and NNS are located throughout the GIT, which may possibly influence appetite. Recent studies investigating the link between hormonal responses in the GI system and being overweight and obese have produced interesting findings. A study by Perry and Wang (219) suggested that these satiety hormonal responses (i.e., PYY, GLP-1 and GIP) are impaired in obese individuals, further raising the likelihood that perhaps energy intake may be poorly regulated among obese individuals due to the impairment in the GI appetite response. (167) Similarly, there is some evidence to suggest that differences in GI hormone secretion exist between lean and obese individuals. (58) For example, the postprandial PYY and GLP-1 responses are attenuated in obese individuals compared to lean individuals, with levels returning to normal following weight loss through gastric bypass surgery. (220-222) Additionally, there is a possibility that obese individuals may experience impaired sweet taste function in comparison to lean individuals, (74) suggesting a coordinated alimentary canal response to sweet taste function from the oral cavity to the GIT. (56)

1.2.6 Conclusions – The Role of Sweet Taste in Satiation and Satiety

Increased energy intake, in particular, increased intake of sweet food, is thought to be one of the contributors to the rising rates in overweight individuals and obesity globally. Based on this idea, one popular way of combating the current obesity crisis is to reduce energy intake through NNS. Replacing sugar with NNS in food and beverages decouples sweetness from the energy value while maintaining sweetness. However, the role of sweet taste in energy intake and appetite regulation is
controversial. The first part of this review focused on discussing the possible associations between sweet taste mechanisms within the oral cavity, GIT and the brain system towards both sugar and NNS. The identification of sweet TRC in both the oral cavity and GIT produced important insights into the mechanisms underlying sweet taste perception for both sugar and NNS. Collectively, the literature investigating the sensing mechanisms of sweet taste detection in both the oral cavity and GIT suggest that perhaps SGLT-1 is more responsible in the gut compared to the oral cavity, where sweet TRC are the primary sugar/NNS sensors. Based on in vivo studies, NNS failed to activate SGLT-1, and thus, no secretion of satiety hormones was observed upon ingestion of NNS in the GIT. It is important to note, however, that this mechanism may not extend to humans, since the studies were animal studies. Therefore, the full description of SGLT-1 in human studies is of relevance due to their apparent involvement in the GI functions and appetite regulation. The available data from brain studies comparing NNS and sugars have also revealed interesting findings regarding habitual consumers and non-habitual consumers of NNS. It was found that compared to a non-habitual consumer of NNS, a habitual consumer of NNS had greater overall activation in the brain reward pathways to both sugar and NNS. The reward pathways of a non-habitual consumer of NNS are generally not activated when consuming NNS as opposed to sugar, suggesting that the NNS may impair and adapt the brain’s capability to detect or sense nutrients. However, it is uncertain at this stage why the brain will adapt to sense NNS as nutrients. It would be interesting for future studies to investigate whether obese habitual users of NNS have impairments in their oral, brain and gut sweetness sensing mechanisms. This would then provide strong evidence linking these mechanisms into a unified theory of obesity development.

It has been suggested that perhaps abnormalities in any or several of these nutrient-sensing mechanisms (i.e. sweet taste detection mechanism in the oral cavity,
the brain and GIT) may be important to understand why some individuals consume more energy. (28) Hence, understanding individual differences in detecting and recognising sweetness towards caloric sugars and NNS and how these mechanisms may influence appetite (sensory-specific satiety and satiety) is important and needs to be verified in the future. Although largely hypothetical at this stage, the review of the literature investigating the potential links between sweet taste function, satiation, satiety and BMI reveals an interesting model and potential factors to understand being overweight and obese.

1.3 The Evidence Supporting the Existence of Oral Complex Carbohydrate Sensitivity

1.3.1 Taste Criteria and Terminology

The prevailing understanding at present is that the human taste system is now widely accepted to include five basic tastes (sweet, sour, bitter, salty, and umami taste), and fat taste being accepted by a few. (55-65) In order for oral perception of complex carbohydrates to be classified as a taste component, certain criteria that have been proposed previously should be met. (61, 223) These criteria comprise the following: 1) provides an adaptive (evolutionary) advantage; 2) is elicited by a unique class of chemicals; 3) has an independent transduction mechanism; 4) signals are detected through gustatory nerves that are processed in the gustatory cortex; 5) is perceptible and has a unique sensation that does not overlap with any other prototypical taste qualities; and 6) raises a behavioural and/or physiological reaction. (61, 223, 224) This review (Section 1.3) will consider the evidence supporting complex carbohydrate as a “taste” component related to each of these criteria. Research on rat’s taste for complex
carbohydrates has previously been critically reviewed in a number of papers (*e.g.* Sclafani (2), Sclafani (8), and Spector & Schier (225)).

The terminology “polysaccharide taste” has been recommended by Sclafani (2) to denote starch-derived saccharides containing three or more glucose units, but this terminology can be confusing as the word “polysaccharide” is generally used to describe complex carbohydrates comprising more than ten monosaccharide units organised in chains. The word “oligosaccharide taste” (two to nine monosaccharide units) would be the more appropriate terminology, however this phrase is not user friendly and it is unknown if perception of oligosaccharides is independent of textural differences. Therefore, at this stage of knowledge, we recommend the use of “oral sensitivity to complex carbohydrate”, which correctly comprises all types of complex carbohydrates and derivatives including fibres (*e.g.* oligofructose), while not diminishing the prospect that oral perception of complex carbohydrates could be due to textural differences. Whilst dietary “carbohydrate” is an umbrella term for the monosaccharide and disaccharide sugars as well as starches and fibres, the term “sweet taste” has been collectively used to indicate sweetness. Thus “oral sensitivity to complex carbohydrate” would at the current state of knowledge be as correct as possible without oversimplifying tasting complex carbohydrates, but not easily confused with other sensations such as sweetness.
1.3.2 Criterion One: Provides an Evolutionary Advantage

One of the many functions of the taste system is to act as a nutrient-toxin detection system. (66) For example, sweet taste indicates the presence of readily available glucose from plant foods, (67) umami taste indicates the presence of proteins, while bitter taste often indicates the presence of poisons. (58, 66) However, it has been suggested recently that sweet taste perception or the degree of sweetness in general is not a good proxy for the amount of energy available in a food. (68, 116) Rather, a detection mechanism encouraging consumption of rich complex carbohydrate foods independent from sweet taste may be advantageous to provide quantitative information about food energy content for functioning (67), especially during times when foods are scarce as complex carbohydrates represent a major source of energy. However, the adaptive advantage of sensing complex carbohydrates could not be verified due to the teleological nature of argument as speculated. (61) For example, it is maladaptive to recognise sweetness from NNS as they do not provide energy for functioning.

1.3.3 Criterion Two: Elicited by a Unique Class of Chemical

One recent study by Lapis et al. (18) proposed that humans may perceive complex carbohydrates (maltodextrin), independent of sweet taste (i.e. glucose, sucrose). Furthermore, the concentrations of glucose and maltose that were inherently present in the complex carbohydrate concentrations tested were mostly at an undetectable range. (18) Generally, in commercially available maltodextrin such as Polycose derived from corn starch, it contains approximately 2% glucose, 7% maltose, and 91% glucose oligomers and polymers. (19) However, Lapis et al. (19) pointed out in a follow up study that it was not clear what substrates can facilitate the detection of glucose oligomers and polymers. In other words, it was uncertain if participants were
able to detect complex carbohydrates because of the glucose oligomers or glucose polymers present in maltodextrin. Interestingly, in the same study by Lapis et al. (19), it was found that humans \((n = 26)\) were able to discriminate glucose oligomer solutions from blank solutions, but not glucose polymer solutions. Furthermore, the participants were able to detect glucose oligomer solutions independent of sweet taste (\(i.e.\) glucose, maltose, sucralose). (19) In this experiment, participants were presented with a set of three stimuli (one target stimulus and two control blanks), and were asked to apply the stimuli across the tip of the tongue with a cotton swab three times with their nose clipped. (72) For the higher concentrations tested, blank water solutions were also prepared with matching viscosities using methylcellulose to account for textural/viscosity cues. (72) It was found that participants were able to discriminate glucose oligomer samples from blank (methylcellulose) samples, but not glucose polymers, thus providing evidence that the stimuli responsible for oral complex carbohydrates sensing in maltodextrin are the glucose oligomers.

1.3.4 Criterion Three: Independent Transduction Mechanism

One obstacle to acceptance of complex carbohydrate as a taste quality has been identification of potential pathways and or receptor(s) for oral complex carbohydrate perception. A significant issue is whether or not complex carbohydrates are detected through the same taste receptor that detects sweetness (\(i.e.\) T1R2-T1R3 heterodimer) (See Section 1.2.3.2 for review on sweet taste detection in the oral cavity).
1.3.4.1 Unidentified Complex Carbohydrate ‘Taste’ Receptor

One of the proposed mechanisms of oral complex carbohydrate is via an unidentified complex carbohydrate taste receptor that responds to complex carbohydrates independently of those of sweet tastants. (8) Two recent human psychophysical studies found that humans were able to perceive complex carbohydrates and that the sensitivity to simple sugar (glucose) is independent of that to complex carbohydrates. (18, 19) For example, Lapis et al. (19) found that humans ($n = 25$) were able to discriminate complex carbohydrate solutions (glucose oligomers) from water even when the sweet taste receptor (T1R2-T1R3 heterodimer) is inhibited by lactisole treatment – a sweet taste blocker known to bind to a pocket in the transmembrane region of the T1R3 and thus inhibits the sweet taste perception of sugars, proteins and NNS. (109) While the human taste perception of complex carbohydrate (starch) hydrolysis products has been well investigated by Lapis et al. (18, 19), it needs replication and also extension. It is important to note, that research which includes taste as a variable generally uses only one measure of taste function or a limited number of concentrations to measure oral sensitivity to complex carbohydrates. In agreement with Lapis et al. (18, 19), there is also mounting evidence indicating that rodents (e.g. rats, hamsters, mice, gerbils) and even some non-human primates are attracted to complex carbohydrates derived from maltodextrin and were able to differentiate both simple and complex carbohydrates apart. Such evidence includes: 1) rodents appear to perceive a distinct taste perception from complex carbohydrates (maltodextrin) that is distinguishable from the taste of simple carbohydrates. (15-17, 226, 227) For example, in a study by Ramirez (227), rodents were trained to avoid corn starch, Polycose (maltodextrin) and sucrose (sweet) aqueous solutions. It was found that rodents trained to avoid maltodextrin containing solutions
avoided maltodextrin at low concentration levels, but continued to consume the starch and sucrose (sweet) solutions. (227) Interestingly, rodents trained to avoid sweet (sucrose) solutions also avoided low concentrations of sucrose, but did not show any aversion behaviours to corn starch or maltodextrin solutions. (227) This study provides evidence that rodents were able to discriminate taste perception for complex carbohydrates from sweetness; (227) 2) knockout rodents missing functional genes for both components of the sweet taste receptor (heterodimer of T1R2 and T1R3) show no hereditary, electrophysiological, or behavioural reactions to simple carbohydrates (glucose, fructose, or sucrose) but respond normally to complex carbohydrates. (15, 228-232) For example, in a study by Treesukosol and Spector, (229) they investigated if T1R2 and T1R3 proteins are responsible for detection of complex carbohydrate stimuli in rodents. In this study, both knockout groups (T1R2 knockout and T1R3 knockout) displayed marked decrease in sensitivity when required to discriminate water from sweet solutions (glucose, maltose, sucrose). (229) However, both knockout groups showed normal values for their psychometric functions when tested with maltodextrin solutions. (229) Thus, this study provides support that the T1R2-T1R3 heterodimer is the primary receptor that mediates sweet taste detection in rodents, but not for complex carbohydrate stimuli; (229) and 3) acceptability of complex carbohydrate (maltodextrin) were found to be unaccounted for by the small amount of free sugars (~0.05-2.88% w/v glucose and maltose) contained in maltodextrin, but rather, rodents appear to be highly attracted to the complex carbohydrate (maltooligosaccharide) itself. (16, 233, 234) Together, these studies raise the potential existence of an unidentified complex carbohydrate taste receptor in humans (235) that responds to complex carbohydrates independently of those of sweet tastants. (8, 225)
1.3.4.2 T1R-Independent Sweet Sensing Pathways

It has also been proposed that the perception of complex carbohydrates can be partly mediated by the T1R-independent sweet sensing pathways in addition to the putative complex carbohydrate receptor. In humans, the primary sweet taste receptor (T1R2-T1R3) has been found to be responsive to caloric sugars (e.g. glucose, fructose, sucrose), NNS (e.g. sucralose, acesulfame K, aspartame), and protein sweeteners (e.g. thaumatin, monellin), but not complex carbohydrates. (232) However, in the absence of T1R2 and T1R3, for example, Tas1r3 KO mice were found to still have significant behavioural and neural responses to caloric sugars suggesting that the sweet taste receptor (T1R2-T1R3 dimer) is not the only sweet sensor in mammalian taste cells. (236) More recent animal studies have hypothesised the presence of a sweet-sensing pathway that is independent of T1R3, a T1R-independent ‘secondary’ mechanism. (108, 236, 237) It has been shown lately that several glucose transporters (GLUTs), ATP-gated K⁺ metabolic sensors, and sodium-glucose co-transporter 1 (SGLT1) are co-expressed in the same sweet-responsive taste cells with T1R3, postulating a possible explanation for the T1R-independent pathway for detecting monosaccharaides. (238, 239) However, the T1R-independent pathways would only explain responses to monosaccharaides (glucose, fructose), but does not on its own, explain the taste responses to disaccharide sugars, such as sucrose and maltose, due to not being substrates for GLUTs or SGLT1. (108, 236) More recent discovery of the taste cell-expressed enzymes such as α-amylase, sucrose-isomaltase, and maltase-glucoamylase indicate that these enzymes may locally break down dietary oligosaccharides, disaccharides, and starch hydrolysis products into monosaccharaides. (236, 240) Thus, these results indicate that the function of these orally expressed enzymes may partly mediate intensity perception of complex
carbohydrates via the T1R-independent sweet pathway in addition to the putative complex carbohydrate detection receptor. (19)

1.3.5 Criterion Four: Signals are Detected through Gustatory Nerves that are Processed in the Gustatory Centres

At this point of time there are no published data on the response of the human’s gustatory nerves to complex carbohydrates. In rodents, one study by Vigorito, Sclafani (241) provided evidence that there is some specialisation of function within the rat’s peripheral gustatory system in response to complex carbohydrates. The results of this study revealed that selective transection of the chorda tympani nerve, glossopharyngeal nerve, greater superficial petrosal nerve, and the pharyngeal branch of the vagus nerve differentially altered the intake of sucrose and maltodextrin solutions. Gustatory denervation of all four gustatory nerves (chorda tympani, glossopharyngeal nerve, greater superficial petrosal nerve, and chorda tympani nerve) in rats decreases their intake of both sucrose and maltodextrin solutions by the same degree. (241) These results show that while the intake of sucrose and maltodextrin seemed to be facilitated to the same level by the gustatory system, the paths involved appear to differ. (2, 241)

1.3.6 Criterion Five: Perceptual Independence

There is only one known human psychophysical study that has investigated if oral sensitivity to complex carbohydrates is independent of some of the other basic tastes (i.e. sweet and salty taste). Lapis et al. (18) showed no significant correlations between the intensity ratings of glucose (sweet taste), sucrose (sweet taste), and
sodium chloride (salty taste) with the intensity ratings of complex carbohydrates. However, at present it is still uncertain if this measure is independent of the intensity ratings of the remaining common prototypical tastes stimuli such as monosodium glutamate (umami taste), caffeine (bitter taste), and citric acid (sour taste). At the intensity (ST) level, it is likely that sensory system other than taste, such as chemesthesis or olfactory are involved. (224) Furthermore, as each measure of taste function (DT, RT, and suprathreshold intensity perception) represents a different dimension of the sense of taste, there is currently no single method to measure taste function in totality. (70) Whether complex carbohydrates are detectable at very low concentrations (i.e. DT), and if complex carbohydrates DTs are independent of DTs for other basic tastes remains to be investigated.

1.3.7 Criterion Six: Behavioural and/or Physiological Responses to Oral Complex Carbohydrate Exposure

Rodents have been found to prefer maltodextrin solutions to water, (6, 16, 241) and they can learn to prefer complex carbohydrate solutions to solutions containing simple sugars especially at low equi-molar concentrations. (6, 17, 242) Furthermore, more recent physiological evidence from exercise studies also supported the notion that humans can detect complex carbohydrates within the oral cavity (see systematic review by Stellingwerff and Cox (243)). For example, Chambers et al. (244) found that exercise performance significantly improved after participants rinsed their mouth with solutions containing complex carbohydrates (maltodextrin) compared to artificially sweetened (NNS) control solutions. These findings were also replicated by other exercise scientists (245-250) raising the potential existence of an unidentified oral complex carbohydrate taste receptor(s) in humans that responds to complex
carbohydrate stimuli independently of those of sweet tastants (see review by Jeukendrup and Chambers (251)). In the second part of the study, Chambers et al. (244) further investigated the corticol response to oral maltodextrin and glucose solutions, revealing a similar pattern of brain activation in response to both solutions, including brain areas believed to be involved in the reward system (i.e. activates brain reward centers in the orbitofrontal cortex and striatum similar to oral glucose, which were unresponsive to NNS). Together, these findings provide strong evidence that there may be taste transduction pathways that respond to complex carbohydrate independently of those for sweet taste. (251)

1.3.8 Possible Functions of Oral Sensitivity to Complex Carbohydrates

A prominent feature of the taste system is the large individual differences in sensitivity to a range of stimuli. (252) Thus, such variation in the taste system along with the role of taste in promoting dietary intake of specific foods or ingredients associated with obesity has been a long area of research but with mixed findings. (55, 56, 63, 158, 179, 181-184) Abnormalities in any or several nutrient receptors are known to influence intake of specific foods or food components related to the nutrient receptor. (253) For example, it is well established that an individual’s sensitivity to bitter tastants (i.e. n-6-propylthiouracil (PROP) and phenylthiocarbamide (PTC)) is determined via genetics, (254) and influences the pleasantness and intake of bitter tasting vegetables such as Brussels sprouts, kale, and asparagus. (253) In addition to an individual’s genetic predisposition, taste sensitivity may also be influenced by environmental factors including a form of adaptive behaviour known as habituation (i.e. decreased response to a stimulus after repeated exposure and consumption of
specific nutrients). (255) To illustrate, this behaviour has been reported for numerous orally detected compounds such as fatty acids, whereby, a negative relationship between habitual fat intake and oral sensitivity to fatty acids has been established. (55, 60) That is, less sensitive individuals towards fatty acids were found to consume more fatty foods. (55, 60) Similarly, after restricting dietary fat intake over a four-week period (57) and six-week period (60), changes in oral sensitivity to fatty acids have also been seen (i.e. more sensitive to fatty acids following decreased intake of dietary fats). A recent study found that oral sensitivity to fatty acids was negatively associated with ad libitum intake of high-fat meals (i.e. satiation or intrameal satiety in response to fat (256)), and in subsequent meal intake (i.e. satiety responses to fat; (257)). Foods high in dietary carbohydrate (simple carbohydrate, complex carbohydrate) have been shown to have a weaker effect on satiation in comparison to other food groups such as dietary protein (257, 258) and result in overconsumption of energy due to their palatability. (259) Furthermore, it has also been reported that there were no significant differences between the satiating effect of sweet and non-sweet complex carbohydrates. (259) If individuals indeed differ in terms of their ability to perceive dietary carbohydrates (oral sensitivity to complex carbohydrates), it is uncertain if an individual’s oral complex carbohydrate sensitivity may influence intake (both habitual and satiation/acute intake) of complex carbohydrates.

1.3.9 Conclusions – The Evidence Supporting the Existence of Oral Complex Carbohydrate Sensitivity

The evidence outlined in the present review provides supports for some of the proposed criteria for a taste component. Nevertheless, due to limited studies conducted in humans, the evidence supporting most of the criteria was not conclusive and this
area requires further research. Although some very interesting findings have been reported by Lapis et al (18, 21) on humans’ perception of complex carbohydrates, at present, there is a pressing need to investigate if the previous human psychophysical studies were reproducible as only single measurements were taken for each testing. Furthermore, there is also a need to understand if oral complex carbohydrates are detectable at very low concentrations, and if complex carbohydrates are perceptually independent from the other basic tastes. Although largely hypothetical at the current time, the associations between oral complex carbohydrate sensitivity, dietary intake, and satiation suggest a fascinating model to understand drivers of complex carbohydrate food consumption.
This thesis comprises nine chapters, which are structured as follows:

**Chapter One** contains an up-to-date review of the literature in this area including background information on overweight and obesity; excess sweet food consumption; the sense of taste and its function; mechanisms for sweet taste detection (oral cavity, gastrointestinal system); non-nutritive sweeteners and their role in the gastrointestinal tract; sweet taste processing in the brain for caloric sweeteners and non-nutritive sweeteners; possible functions of sweet taste perception; implications of decreased oral and gastrointestinal sweet taste sensitivity and perception; the evidence supporting the existence of oral complex carbohydrate sensitivity as a “taste” component; the possible functions of oral sensitivity to complex carbohydrates, as well as a brief review of satiation and the psychophysics methodology used in human complex carbohydrate studies, what it tells us, and how it relates to taste and consumption.

**Chapter Two** outlines all methodology used in this research, which comprised three sensory evaluation studies. **Chapters Three to Eight** detail the three studies (each study is presented in two chapters) and include introduction, methods, results, discussion and conclusion for each. **Study 1(a) (Chapter Three)** is a comprehensive psychophysical evaluation of sweetness functions across multiple sweeteners. **Study 1(b) (Chapter Four)** investigated the association between sweet taste function, body composition, and dietary intake in adults. **Study 2(a) (Chapter Five)** is a comprehensive investigation of oral complex carbohydrate sensitivity in humans. **Study 2b (Chapter Six)** investigated the associations between oral complex carbohydrate sensitivity, body composition, and dietary intake in adults. **Study 3(a) (Chapter Seven)** was a psychophysics study involving
participation of 132 adults that investigated the associations between sweet taste function and oral complex carbohydrate sensitivity, BMI, liking, and consumption of sweet and carbohydrate prototypical foods. Using the same participants from Study 3(a), **Study 3(b) (Chapter Eight)** investigated the associations between sweet taste function and oral complex carbohydrate sensitivity, liking, and consumption of *ad libitum* sweet and carbohydrate milkshakes. Finally, **Chapter Nine** summarises the findings of the three studies conducted, their limitations, and future directions for research.
1.4 Thesis aim, objectives, and hypotheses

1.4.1 Overall Aim and Objectives

The aim of this thesis was to assess whether individual variations in sweet taste function and oral complex carbohydrate sensitivity may influence body composition and dietary intake (both habitual and acute intake). A secondary aim was to investigate the psychophysics of sweet taste function and oral complex carbohydrate sensitivity across a range of sweet tastants and complex carbohydrates. More precisely, across the three studies, the objectives were to:

- Investigate the three main measures of sweet taste function – detection threshold, recognition threshold, and suprathreshold intensity – across a range of caloric and non-nutritive sweeteners;
- Investigate the associations between the three common measures of sweet taste function using multiple sweeteners, body composition, and dietary intake among adults;
- Investigate if humans can perceive two complex carbohydrates including a soluble starch, maltodextrin, and a soluble fibre, oligofructose;
- Investigate associations between oral complex carbohydrate sensitivity (maltodextrin, and a soluble fibre oligofructose), body composition, and dietary intake among adults;
- Confirm if humans can perceive complex carbohydrate from a range of concentration levels; and if oral sensitivity to complex carbohydrate relates to BMI, liking, and consumption of carbohydrate foods in a larger sample group of adults;
Chapter 1 – Literature Review

- Measure if sweet taste function and oral sensitivity to complex carbohydrates relates to *ad libitum* consumption of sweet and complex carbohydrate milkshakes in a sample group of adults.

### 1.4.2 Hypotheses

This thesis will test the following hypotheses:

- There will be significant individual variance in sweet taste sensitivities for detection and recognition thresholds. There will also be significant individual variance in perceived sweetness intensity. Participants will be able to be classified into more sensitive/experienced high intensity, normal sensitive/experienced moderate intensity, and less sensitive/experienced low intensity groups according to the sweetness of various sweeteners, and sweet taste measures;

- No measure of sweet taste function (detection threshold, recognition threshold, or suprathreshold intensity perception) will be associated with body composition and dietary intake for all sweeteners;

- There will be an association between sweet taste suprathreshold intensity and satiation, but no association between sweet taste detection threshold and satiation;

- Humans will have detection threshold and suprathreshold intensity for complex carbohydrates;

- There will be significant individual variance in oral complex carbohydrate sensitivity. Participants will be able to be classified into more sensitive/experienced high intensity, normal sensitive/experienced moderate
intensity, and less sensitive/experienced low intensity groups according to the oral sensitivities of two complex carbohydrates (soluble starch maltodextrin and soluble fibre oligofructose);

- There will be an association between oral complex carbohydrate sensitivity (suprathreshold intensity only), body composition, and dietary intake, but no association between oral complex carbohydrate sensitivity (detection threshold), body composition, and dietary intake;

- There will be an association between oral complex carbohydrate sensitivity (suprathreshold intensity only) and satiation, but no association between oral complex carbohydrate sensitivity (detection threshold) and satiation.
Chapter Two: Materials, Methodology, and Measurements

Components of this chapter have been published in Chemical Senses (2016): bjw109 as 'Psychophysical Evaluation of Sweetness Function Across Multiple Sweeteners' and in Nutrients, 8(4), 241 as 'The Association between Sweet Taste Function, Anthropometry, and Dietary Intake in Adults'

2.1 Introduction

Common materials, methods, and measurements, including all sensory testing procedures used throughout this thesis are described in this chapter. The methodology is also briefly outlined within each respective chapter. All methods and techniques used to complete this thesis were well established within the Sensory Laboratory at Deakin University, Burwood, Australia.

2.2 Participants

2.2.1 Participant Demographics

All participants for Study 1 and Study 2 were screened prior to study enrolment for eligibility (Appendix A). Participants were not eligible if they: (1) smoked; (2) were pregnant or lactating; (3) were taking any prescription medication that may interfere with their ability to taste; or (4) had a history of food allergies that may interfere with these studies. Demographic information including age and gender were also collected. Participants were recruited from locations adjacent to the Melbourne Burwood campus via email and flyer distribution (Appendix B and Appendix C). For Study 3, participants were recruited from a convenience sample of 138 students enrolled in a third-year Sensory Evaluation of Foods unit during 2016 at
Deakin University, Melbourne campus, Australia. A total of 132 participants gave written informed consent and participated in the study. However, data were excluded for individuals who: (1) were smokers; (2) were pregnant or lactating; (3) were taking any prescription medication that may interfere with their ability to taste; (4) had a history of any food allergy that may interfere with the study. Demographic information including age and gender were also collected (Appendix D). Participants in all studies were aged between 18 – 55 years at the time of testing. For all studies, participants were in good health. For **Study 1** and **Study 2**, a sample size of more than 30 participants was chosen to approximate a normal population distribution according to the central limit theorem. (260) For **Study 3**, a sample size of more than 49 participants was determined using knowledge from prior literature and is detailed within **Chapter 8**.

### 2.2.2 Reimbursement

Reimbursements were provided in the form of a Coles Myer Group and WISH (supermarket/department stores) gift card. A $160 reimbursement was provided for **Study 1** and a $100 gift card for **Study 2**. No reimbursement was provided for those who participated in **Study 3**.

### 2.3 Ethics

All studies were conducted according to the guidelines laid down by the Declaration of Helsinki. The Deakin University Human Research Ethics Committee and Deakin University Health Ethics Committee approved all the procedures involving human participants prior to study commencement. (approval numbers listed in chapters for individual studies). All three studies were registered at [www.anzctr.org.au](http://www.anzctr.org.au)
as clinical trials (approval numbers listed in chapters for individual studies). Written informed consent was obtained from all participants prior to participation and participants were free to withdraw from all studies at any stage throughout the course of the study.

2.4 Sensory Testing

2.4.1 Sensory Testing
All sensory tasks (except hedonic ratings for sweet and complex carbohydrate based foods) were conducted in computerised, partitioned sensory booths in the Centre for Advanced Sensory Science using Compusense Five Software Version 5.2 (Study 1) (Compusense Inc., Ontario, Canada) or Compusense Cloud Software as part of the Compusense Academic Consortium (Study 2 and Study 3) (Compusense Inc., Ontario, Canada). Hedonic ratings for a range of sweet and complex carbohydrate based foods were conducted in individual workbenches at our teaching laboratory. Participants were asked to refrain from eating, drinking (except water), or chewing gum for at least one hour prior to testing.

2.4.2 Sweet Taste Solutions – Materials and Methods
Both caloric [glucose monohydrate (The Melbourne Food Depot, Melbourne, Australia), fructose (The Melbourne Food Depot, Melbourne, Australia); sucrose (CSR, Yarraville, Australia), erythritol (AuSweet, Melbourne, Australia)] and non-nutritive sweeteners (NNS) [sucralose (The Melbourne Food Depot, Melbourne, Australia), Rebaudioside A (AuSweet, Melbourne, Australia)] were used to investigate sweet taste function [detection threshold (DT), recognition threshold (RT), and suprathreshold intensity perception (ST)] in Study 1 and Study 2 (only DTs and STs).
Chapter 2 – Materials, Methodology, and Measurements

For **Study 3**, glucose monohydrate (The Melbourne Food Depot, Melbourne, Australia) was used to investigate sweet taste function (DT and ST only).

The concentration series for sucrose DT and RT was adapted from ISO3972. (261) The concentration series for glucose monohydrate, fructose, sucralose, Rebaudioside A, and erythritol DTs and RTs were prepared in successive 0.25 log dilution steps. Initial starting concentrations were determined through informal bench-top testing, based on modified findings of matching sweetness intensity ratios published by Keast *et al.* (262). Detailed in **Table 2.1** are the concentration ranges used to assess DTs and RTs for sweet taste in **Study 1** and **Study 2** (DTs only). **Table 2.2** gives the nine concentrations used to assess DT for sweet taste in **Study 3** (glucose monohydrate; the ninth concentration being presented only when participants were unable to detect a difference from water solution in the previous eight). Three concentrations (weak, medium, and strong) and a control (blank) solution were prepared to determine perceived suprathreshold intensity for each sweetener (glucose monohydrate only for **Study 3**; **Table 2.3**). These concentrations were derived through informal bench-top testing (ascending taste intensity) and were similar to the concentrations outlined by Webb *et al.* (70). The concentrations for each prototypical stimulus ranged from ‘weak’ to ‘strong’ on the gLMS.

To prepare the solution, the appropriate amount of each sweetener was weighed (Pioneer OHAus Corporation, Pine Brook, USA) and added individually to 1 L of deionised filtered water (Cuno Filter Systems FS117S, Meriden, CT, USA). Solutions were stirred on a stirring plate to allow each of the compounds to dissolve and produce the relevant concentrated solutions. All samples were freshly prepared on each day of testing and stored in glass beakers at room temperature (20 ± 1 °C).
2.4.3 Sweet Taste Solutions – Threshold Measurements

For Study 1, DTs for each of the sweeteners were determined using ascending forced choice triangle methodology (Figure 2.1), (263, 264) in which the participants were provided with three 25 mL samples, two of which were controls (filtered deionised water) and one containing sweetener, in ascending order from the lowest to the highest concentration. The order in which samples were presented was randomised throughout the procedure. For example, if the control samples were labelled A and the sweetened samples as B, samples could be presented as AAB, BAA, or ABA. (263) Participants were instructed to place a small amount of solution in their mouth, swirl the sample around in their mouth for five seconds, and then expectorate. While the sample was in their mouth, participants were asked to concentrate on a specific taste quality (sweet taste in this case) of that sample. Participants were also advised to start tasting from the left of the tray and move through to the right until all of the samples had been tasted. Once they had tried all of the samples, they were able to go back through the samples until they thought they could identify the odd sample (sweetened sample). DT was defined as the concentration of sweetener required for a participant to correctly identify the sweetened sample as the odd one out in three consecutive sample sets at one concentration level (Appendix E). (263)
Table 2.1 Sweetener concentrations used for determination of detection and recognition thresholds in Study 1 and Study 2. *

<table>
<thead>
<tr>
<th>Sweetener</th>
<th>Concentration (% w/v)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose monohydrate</td>
<td></td>
<td>0.02</td>
<td>0.03</td>
<td>0.05</td>
<td>0.09</td>
<td>0.1</td>
<td>0.2</td>
<td>0.4</td>
<td>0.6</td>
<td>1.1</td>
<td>1.8</td>
<td>2.9</td>
<td>4.8</td>
</tr>
<tr>
<td>Fructose</td>
<td></td>
<td>0.01</td>
<td>0.02</td>
<td>0.03</td>
<td>0.05</td>
<td>0.08</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.5</td>
<td>0.9</td>
<td>1.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Sucrose</td>
<td></td>
<td>0.01</td>
<td>0.02</td>
<td>0.03</td>
<td>0.06</td>
<td>0.09</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.7</td>
<td>1.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Sucralose (x10^{-3})</td>
<td></td>
<td>0.02</td>
<td>0.04</td>
<td>0.06</td>
<td>0.09</td>
<td>0.1</td>
<td>0.2</td>
<td>0.4</td>
<td>0.7</td>
<td>1.1</td>
<td>1.9</td>
<td>3.1</td>
<td>5.1</td>
</tr>
<tr>
<td>Rebaudioside A (x10^{-3})</td>
<td></td>
<td>0.03</td>
<td>0.05</td>
<td>0.09</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.6</td>
<td>1.0</td>
<td>1.7</td>
<td>2.8</td>
<td>4.6</td>
<td>7.7</td>
</tr>
<tr>
<td>Erythritol</td>
<td></td>
<td>0.02</td>
<td>0.03</td>
<td>0.05</td>
<td>0.08</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.6</td>
<td>0.9</td>
<td>1.6</td>
<td>2.6</td>
<td>4.4</td>
</tr>
</tbody>
</table>

* Only detection thresholds were determined in Study 2.

The concentration series for sucrose was adapted from ISO3972. (261) The concentration series for glucose monohydrate, fructose, sucralose, Rebaudioside A, and erythritol were prepared in successive 0.25 log dilution steps. Reference chemical details: glucose monohydrate (The Melbourne Food Depot, Melbourne, Australia); fructose (The Melbourne Food Depot, Melbourne, Australia); sucrose (CSR, Yarraville, Australia); sucralose (The Melbourne Food Depot, Melbourne, Australia); Rebaudioside A (AuSweet, Melbourne, Australia); and erythritol (AuSweet, Melbourne, Australia).
Table 2.2 *Sweetener and complex carbohydrate concentrations used for determination of detection thresholds in Study 3.*

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Concentration (% w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Glucose monohydrate</td>
<td>0.05</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Amount of Glucose in Maltodextrin (×10^-3)

|                        | 0.9   | 1.8   | 2.7   | 5.4   | 9.9   | 17.1   | 32.4   | 56.7   | 100.8 |

Amount of Total Sugars in Maltodextrin (×10^3)

|                        | 1.7   | 3.4   | 5.1   | 10.2  | 18.7  | 32.3   | 61.2   | 107.1  | 190.4 |

The concentration series for glucose monohydrate and maltodextrin were prepared with successive 0.25 log dilution steps. Reference chemical details: glucose monohydrate (The Melbourne Food Depot, Melbourne, Australia); maltodextrin (Star-Dri 5, Tate & Lyle Ingredients Americas, USA). The ninth concentration was presented only when participants were unable to detect a difference from water solution in the previous eight. (70) Calculation of the amount of common and total sugars in maltodextrin concentrations were according to the report of analysis by the Australian Government National Measurement Institute from samples used in this study, where there were a total of 1.7g/100g (1.7% w/w) of free sugars for the maltodextrin (Glucose: 0.9% w/w).
### Table 2.3 Concentrations (weak, medium, and strong intensity) of sweeteners used for determination of suprathreshold taste intensity in Study 1, Study 2, and Study 3.*

<table>
<thead>
<tr>
<th>Sweetener</th>
<th>Concentration (% w/v)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weak</td>
<td>Medium</td>
<td>Strong</td>
<td></td>
</tr>
<tr>
<td>Glucose monohydrate</td>
<td>5.3</td>
<td>10.6</td>
<td>21.2</td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>2.9</td>
<td>5.6</td>
<td>11.2</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>3.4</td>
<td>6.9</td>
<td>13.7</td>
<td></td>
</tr>
<tr>
<td>Sucralose (x10^{-3})</td>
<td>5.7</td>
<td>11.4</td>
<td>22.8</td>
<td></td>
</tr>
<tr>
<td>Rebaudioside A (x10^{-3})</td>
<td>8.6</td>
<td>17.2</td>
<td>34.4</td>
<td></td>
</tr>
<tr>
<td>Erythritol</td>
<td>5.7</td>
<td>9.8</td>
<td>19.7</td>
<td></td>
</tr>
</tbody>
</table>

* For Study 3, only glucose monohydrate was used to investigate suprathreshold taste intensity.
Figure 2.1 Flow diagram of the ascending forced choice triangle methodology. (263, 264) As shown, participants began with the lowest concentration of a tastant (e.g. 0.05% w/v glucose monohydrate), along with two control samples. Participants were instructed to select the ‘odd’ one out which contained a fixed concentration of particular sweetener/complex carbohydrate. If a participant was incorrect, a second sample set with the next highest concentration of sweetener/complex carbohydrate was presented (e.g. 0.09% w/v). However, if correct, a second set was presented with the same concentration as the preceding tray. This continued until the participant could identify the odd sample correctly three consecutive times.
Chapter 2 – Materials, Methodology, and Measurements

For **Study 3**, DT was determined using the procedure outlined in the International Standards Organisation (ISO) Method of Investigating Sensitivity of Taste. (265) The eight samples for glucose were served in ascending concentration (15 mL per sample, in accordance with the standard ISO method). Participants were unaware of the presentation order and were instructed to taste each sample for five seconds then expectorate and rate whether: there was an absence of taste (water-like); or if a taste was identified but not recognised. (261) DT was defined as the concentration at which the participants selected the ‘taste identified, but unknown taste quality’ ([Appendix H](#)). (261) Participants were navigated through the procedure by both written (on the computer screen) and verbal instructions (read from a scripted paper) from the researcher.

The RTs (**Study 1**) for each of the sweeteners were measured using a whole-mouth, sip-and-spit procedure. (76) Each participant received a single 15 mL sample, presented in a medicine cup, in ascending order starting from his or her DT concentration level. Participants were asked to identify the quality of the taste after holding the sample in their mouth for at least five seconds. Response options included ‘sweet’, ‘sour’, ‘bitter’, ‘salty’, ‘umami’ or ‘unknown taste’. Participants tasted each sample once, in ascending concentration order, until they identified the target taste quality ‘sweet’ for all of the sweeteners. (76) RT was defined as the concentration at which they were able to recognise the correct taste quality three times consecutively. To prevent participants from learning the purpose of the task, they were told that the purpose of this experiment was to investigate if they were able to detect any other potential taste qualities before the final ‘sweet’ perception. They were also given examples of how some people were able to detect other taste qualities such as bitterness when tasting NNS. We found that this strategy encouraged participants to attempt recognition (not only sweet) prior to concentrations associated with
probabilistic recognition (*i.e.* the concentrations at which participants were able to recognise quality imperfectly at above chance level). (76) At the end of the final visit, participants were debriefed about the experiment, and none was aware that the purpose of this task was a sham.

Filtered deionised water was used as an oral rinsing agent. Participants were instructed to rinse their mouths with filtered deionised water for five seconds before beginning each task and between each sample set. To eliminate any visual and olfactory input, all sensory testing sessions were conducted under red lighting, and participants were asked to wear nose clips during testing. All solutions were served at room temperature, with a three-digit code allocated to each sample. Random three-digit codes were generated using a random number generator website (www.randomizer.org).

### 2.4.4 Sweet Taste Solutions – Intensity Measurements

#### 2.4.4.1 Participant Training

Prior to using the general Labeled Magnitude Scale (gLMS) to rate taste intensity, participants were trained using the standard protocol outlined by Green *et al.* (266, 267) except the top of the scale was described as the strongest imaginable sensation of any kind (*Appendix G*). (75) The 100-point scale comprised the following adjectives: ‘no sensation’ = 0, ‘barely detectable’ = 1.5, ‘weak’ = 6, ‘moderate’ =17, ‘strong’ = 35, ‘very strong’ = 52, and ‘strongest imaginable’ = 100. (75) Only scales with adjectives were presented to participants (no equivalent numbers, although numerical data were extracted from the scale for data analysis). (70) During the training session, participants were asked to rate the intensity of the perceived sensation relative to a remembered or imagined sensation. Participants were
required to rate a list of seven remembered or imagined sensations, such as the warmth of lukewarm water, the pain from biting of the tongue, and the sweetness of fairy floss (known as cotton candy in the USA, or candy floss in the UK) (Appendix H).

### 2.4.4.2 Standardisation of gLMS Usage with Weight Ratings

To standardise gLMS usage within participants, a modified version of Delwiche et al. (268) was adapted for this study. To control for idiosyncratic scale usage, participants were asked to rate the heaviness of six, visually identical weights (opaque bottles filled with sand and stone and completely wrapped in aluminium foil; weights of 53, 251, 499, 724, 897, and 1127g) (Appendix I). Participants were asked to hold out their non-dominant hand palm up, while the experimenter placed the weighted bottle on the palm of the hand. Participants were instructed to rate the heaviness of each weight using the gLMS.

As detailed in each study, correlations between the overall prototypical intensity ratings and overall mean heaviness ratings were calculated. Assuming that the intensity ratings of prototypical ratings and the heaviness of the bottles were unrelated, if significant correlations were observed, it indicates that the gLMS ratings were subject to differences in individual scale-use and thus require standardisation across participants. (54, 70, 268) To determine a personal standardisation factor, the grand mean for heaviness across weight levels and participants was divided by each participant’s average intensity for heaviness. (54) Each individual’s sweet taste intensity ratings were multiplied by his or her personal standardisation factor for scale-use bias. (54, 268)
2.4.4.3 Sweet Taste Intensity Measurement Procedures

Trays containing three concentrations (weak, medium, and strong) and a control (blank) solution were prepared to determine perceived ST for sweeteners. Each stimulus was presented to participants independently (sets), but in a randomised order. Prior to tasting the samples, participants were instructed to rinse their mouth with filtered deionised water prior to sampling the set of solutions. The solutions were presented with a randomised three-digit code, and tastings were conducted under red lights. Wearing a nose clip, participants were instructed to place the 15 mL samples into their mouth, hold the sample in their mouth for 5 seconds, and expel it. Participants were required to rate the sweet ST on the gLMS. Participants were also asked to rinse their mouth for 5 seconds with deionised water between samples.

2.4.5 Oral Complex Carbohydrate Sensitivity Solutions – Materials and Methods

Maltodextrin (Star-Dri 5, Tate & Lyle Ingredients Americas, USA) and oligofructose (Fibrulose F97, CoSucra-Groupe Warcoing, Belgium) were used to investigate oral complex carbohydrate sensitivity (DTs and STs for both complex carbohydrates) in Study 2. For Study 3, only maltodextrin (Star-Dri 5, Tate & Lyle Ingredients Americas, USA) was used to investigate oral complex carbohydrate sensitivity (DT and ST). Maltodextrin with a dextrose equivalent (DE) of five was used in this study as it contains the lowest possible amount of free sugar (glucose, maltose) yet is soluble in water (see Section 2.4.5.1 on analysis of common sugars in maltodextrin samples). DE is a measure of the percentage of reducing sugars relative to glucose on a dry basis. (269) Detailed in Table 2.4 are the concentration ranges used to assess DT for oral complex carbohydrate sensitivity in Study 2. The
concentration series for complex carbohydrates were prepared with successive 0.25 log dilution steps. (270)

Concentrations for complex carbohydrates (maltodextrin, oligofructose) were derived based on previously published findings of perceptually distinctive oral sensation concentrations (i.e. see Lapis et al. (18) and systematic literature review by e Silva (271)) without perceivable viscosity. After pilot testing, a concentration range between 0.04-20.0 percent (w/v) was used to measure DT levels for complex carbohydrates. As maltodextrin is similar in oral sensation and appearance to oligofructose, similar concentrations were used for both complex carbohydrates. (272-275) Table 2.2 gives the nine concentrations used to assess DT for oral complex carbohydrate sensitivity in Study 3 (maltodextrin; the ninth concentration being presented only when participants were unable to detect a difference from water solution in the previous eight). A concentration range between 0.1-11.2 percent (w/v) was used to measure DT levels for oral complex carbohydrate sensitivity in Study 3.

Four concentrations of complex carbohydrate solutions (weak, medium, medium-strong, strong) and a control (blank) solution were prepared (Table 2.5) to determine perceived suprathreshold intensity for oral complex carbohydrate sensitivity for each complex carbohydrate in Study 2. Three concentrations (3.6, 6.3, and 11.2 % w/v) and a control (blank) solution were prepared to determine perceived suprathreshold intensity for maltodextrin in Study 3. These concentrations were derived through informal bench-top testing (ascending taste intensity).
### Table 2.4 Complex carbohydrate concentrations used for determination of detection thresholds in Study 2.

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Concentration (% w/v)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltodextrin</td>
<td></td>
<td>0.04</td>
<td>0.06</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.6</td>
<td>1.1</td>
<td>1.9</td>
<td>3.6</td>
<td>6.3</td>
<td>11.2</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>Amount of Glucose in Maltodextrin (x10^-3)</td>
<td>0.3</td>
<td>0.5</td>
<td>0.9</td>
<td>1.6</td>
<td>2.8</td>
<td>5.8</td>
<td>9.0</td>
<td>15.9</td>
<td>28.4</td>
<td>50.5</td>
<td>90.0</td>
<td>160.0</td>
</tr>
<tr>
<td></td>
<td>Amount of Total Sugars in Maltodextrin (x10^-3)</td>
<td>1.1</td>
<td>1.6</td>
<td>3.0</td>
<td>5.6</td>
<td>9.8</td>
<td>17.6</td>
<td>31.4</td>
<td>55.7</td>
<td>99.4</td>
<td>176.7</td>
<td>314.7</td>
<td>560.0</td>
</tr>
<tr>
<td>Oligofructose</td>
<td></td>
<td>0.04</td>
<td>0.06</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.6</td>
<td>1.1</td>
<td>1.9</td>
<td>3.6</td>
<td>6.3</td>
<td>11.2</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>Amount of Fructose in Oligofructose (x10^-3)</td>
<td>0.5</td>
<td>0.8</td>
<td>1.5</td>
<td>2.8</td>
<td>4.9</td>
<td>8.8</td>
<td>15.6</td>
<td>27.8</td>
<td>49.7</td>
<td>88.3</td>
<td>157.3</td>
<td>280.0</td>
</tr>
<tr>
<td></td>
<td>Amount of Total Sugars in Oligofructose (x10^-3)</td>
<td>1.2</td>
<td>1.8</td>
<td>3.3</td>
<td>6.6</td>
<td>10.5</td>
<td>18.9</td>
<td>33.6</td>
<td>59.7</td>
<td>106.5</td>
<td>189.3</td>
<td>337.2</td>
<td>600.0</td>
</tr>
</tbody>
</table>

The concentration series for maltodextrin and oligofructose were prepared with successive 0.25 log dilution steps. Reference chemical details: maltodextrin (Star-Dri 5, Tate & Lyle Ingredients Americas, USA) and oligofructose (Fibrulose F97, CoSuca-Groupe Warcoing, Belgium). Calculation of the amount of common and total sugars in maltodextrin and oligofructose concentrations were according to the report of analysis by the Australian Government National Measurement Institute from samples used in this study, where there were a total of 2.8g/100g (2.8% w/w) of free sugars for the maltodextrin (Glucose: 0.8% w/w) and 3.0g/100g (3.0% w/w) of free sugars for the oligofructose (Fructose: 1.4% w/w).
Table 2.5 Concentrations (weak, medium, medium-strong, and strong intensity) of complex carbohydrates used for determination of suprathreshold intensity in Study 2.

<table>
<thead>
<tr>
<th>Concentration (% w/v)</th>
<th>Weak</th>
<th>Medium</th>
<th>Medium-Strong</th>
<th>Strong</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltodextrin</td>
<td>3.6</td>
<td>6.3</td>
<td>11.2</td>
<td>20.0</td>
</tr>
<tr>
<td>Oligofructose</td>
<td>3.6</td>
<td>6.3</td>
<td>11.2</td>
<td>20.0</td>
</tr>
</tbody>
</table>

To prepare the solution, the appropriate amount of each complex carbohydrate was weighed (Pioneer OHAus Corporation, Pine Brook, USA) and added individually to 1 L of deionised filtered water (Cuno Filter Systems FS117S, Meriden, CT, USA). Solutions were stirred on a stirring plate to allow each of the compounds to dissolve and produce the relevant concentrated solutions. All samples were freshly prepared on each day of testing and stored in glass beakers at room temperature (20 ± 1 °C).

2.4.5.1 Analysis of Common Sugars in Maltodextrin and Oligofructose Samples

To determine if the maltodextrin and oligofructose used in Study 2 and Study 3 would be suitable products, four percent w/v maltodextrin and oligofructose solutions were prepared for High Performance Liquid Chromatography (HPLC) (Appendix J). The complex carbohydrate extracts were clarified with 25 mL acetonitrile and filtered through a 0.45 um filter into a 2 mL vial. To determine the amount of common sugars in samples, filtered solutions were analysed by HPLC using amino column with an acetonitrile/water mobile phase containing salt and refractive index detection. Quantitation was made using a standard solution containing known amounts of fructose, glucose, sucrose, maltose and lactose. Samples were measured in duplicate.
Chapter 2 – Materials, Methodology, and Measurements

For Study 2, there were a total of 2.8g/100g (2.8% w/w) of free sugars for the maltodextrin (Glucose: 0.8% w/w) and 3.0g/100g (3.0% w/w) of free sugars for the oligofructose (Fructose: 1.4% w/w) used in this study. Detailed in Table 2.4 are the amounts of common sugars and total sugars (% w/v) present in each complex carbohydrate DT concentration. For Study 3, there were a total of 1.7g/100g (1.7% w/w) of common sugars for the maltodextrin used in this study (Glucose: 0.9% w/w). Detailed in Table 2.2 are the amount of glucose and total sugars (% w/v) present in each maltodextrin DT concentration.

2.4.6 Oral Complex Carbohydrate Sensitivity Solutions – Threshold Measurements

In Study 2, DTs for each of the complex carbohydrates were determined using ascending forced choice triangle methodology (Figure 2.1), (263, 264) in which the participants were provided with sets of three 25 mL samples, two of which were controls (filtered deionised water) and one contained complex carbohydrate, in ascending order from the lowest to the highest concentration level. DT was defined as the concentration of complex carbohydrate required for a participant to correctly identify the complex carbohydrate sample, as the odd one out in three consecutive sample sets at one concentration level. (263) In Study 3, eight samples for complex carbohydrate (maltodextrin) were served in ascending concentration (15 mL per sample, in accordance with the standard ISO method). Participants were unaware of the presentation order and were instructed to taste each sample for five seconds then expectorate and rate whether: there was an absence of oral perception (water-like); or if an oral perception was identified but not recognised. (261) DT was defined as the
concentration at which the participants selected the ‘oral perception identified, but unknown oral perception’. (261)

Filtered deionised water was used as an oral rinsing agent. Participants were instructed to rinse their mouths with filtered deionised water for five seconds before beginning each task and between each sample set. To eliminate any visual and olfactory input, all sensory testing sessions were conducted under red lighting, and participants were asked to wear nose clips during testing. All solutions were served at room temperature, with a three-digit code allocated to each sample.

2.4.7 Oral Complex Carbohydrate Sensitivity Solutions – Intensity Measurements

2.4.7.1 Participant Training and Standardisation of gLMS with Weight Ratings

The training procedures prior to using the gLMS to rate oral intensity of complex carbohydrate solutions were similar to the procedures outlined previously in Section 2.4.4.1. Likewise, the standardisation procedures of gLMS usage within participants with weight ratings for complex carbohydrate solutions were similar to the procedures outlined in Section 2.4.4.2.

2.4.7.2 Oral Complex Carbohydrate Sensitivity - Intensity Measurement Procedures

For Study 2, trays containing four concentrations of complex carbohydrate solutions (weak, medium, medium-strong, strong) and a control (blank) solution were prepared to determine perceived ST for complex carbohydrates (maltodextrin,
Chapter 2 – Materials, Methodology, and Measurements

Oligofructose. Each complex carbohydrate was presented to participants independently (sets), but in a randomised order. For Study 3, trays containing three concentrations of complex carbohydrate solutions (weak, medium, strong) and a control (blank) solution were prepared to determine perceived ST for complex carbohydrate (maltodextrin). Prior to tasting the samples, participants were instructed to rinse their mouth with filtered deionised water prior to sampling the set of solutions. The solutions were presented with a randomised three-digit code, and tastings were conducted under red lights. Wearing a nose clip, participants were instructed to place the 15 mL samples into their mouth, hold it in their mouth for 5 seconds, and expectorate. Participants were required to rate the oral intensity of the samples on the gLMS. Participants were also asked to rinse their mouth for 5 seconds with deionised water between samples.

2.4.8 Five Primary Tastes – Materials and Methods

Prototypical stimuli [sucrose (CSR, Yarraville, Australia), sodium chloride (Saxa, Premier Foods Inc, Seven Hills, Australia), caffeine (Sigma Aldrich, Steinham, Germany), citric acid (Ward McKenzie Private Limited, Altona, Australia), and monosodium glutamate (MSG; Ajinomoto Cooperation, Tokyo, Japan)] were used to investigate taste function for the five basic tastes in Study 1 and Study 2. DTs and RTs for the five primary tastes were determined using the procedure outlined in the International Standards Organisation (ISO) Method of Investigating Sensitivity of Taste (only DTs of salty, sour, bitter, and umami taste were determined in Study 2).
Table 2.6 gives the nine concentrations used for each taste quality (the ninth concentration being presented only when participants were unable to recognise the taste quality in the previous eight). (265) Three concentrations (weak, medium, and strong) and a control (blank) solution were prepared to determine perceived suprathreshold intensity for each prototypical tastant (Table 2.7). These concentrations were derived through informal bench-top testing (ascending taste intensity) and were similar to the concentrations outlined by Webb et al. (70).

To prepare the solution, the appropriate amount of each stimuli was weighed (Pioneer OHAus Corporation, Pine Brook, USA) and added individually to 1 L of deionised filtered water (Cuno Filter Systems FS117S, Meriden, CT, USA). Solutions were stirred on a stirring hotplate to allow each of the compounds to dissolve and produce the relevant concentrated solutions. All samples were freshly prepared on each day of testing and stored in glass beakers at room temperature (20 ± 1 °C).
Table 2.6 Stimulus concentrations used for prototypical threshold testing in Study 1 and Study 2.*

<table>
<thead>
<tr>
<th>Taste quality</th>
<th>Stimulus</th>
<th>Concentrations (% w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Sweet</td>
<td>Sucrose</td>
<td>0.03</td>
</tr>
<tr>
<td>Salty</td>
<td>Sodium chloride</td>
<td>0.01</td>
</tr>
<tr>
<td>Sour</td>
<td>Citric acid</td>
<td>0.013</td>
</tr>
<tr>
<td>Bitter</td>
<td>Caffeine</td>
<td>0.006</td>
</tr>
<tr>
<td>Umami</td>
<td>Monosodium glutamate</td>
<td>0.008</td>
</tr>
</tbody>
</table>

* Only DTs of salty, sour, bitter, and umami taste qualities were determined in Study 2.

The concentration series were adapted from ISO3972. (261) Reference chemical details: sucrose (CSR, Yarraville, Australia); sodium chloride (Saxa, Premier Foods Inc, Seven Hills, Australia); caffeine (Sigma Aldrich, Steinham, Germany); citric acid (Ward McKenzie Private Limited, Altona, Australia); and monosodium glutamate (Ajinomoto Cooperation, Tokyo, Japan).
Chapter 2 – Materials, Methodology, and Measurements

2.4.9 Five Primary Tastes – Threshold Measurements

DTs and RTs were determined using the procedure outlined in the International Standards Organisation (ISO) Method of Investigating Sensitivity of Taste. (265) A tray was served containing eight samples from each taste quality in ascending concentration (15 mL per sample, in accordance with the standard ISO method), and each taste quality was presented to participants independently. Participants were unaware of the presentation order but were informed of the possible taste qualities. Participants were instructed to taste each sample for five seconds then expectorate and record whether: there was an absence of taste (water-like); a taste was identified but not recognised; or a taste quality was perceived. (265) DT was defined as the concentration at which the participants selected the ‘taste identified, but unknown taste quality’ response (Appendix K). (265)

Filtered deionised water was used as an oral rinsing agent. Participants were instructed to rinse their mouth with filtered deionised water for five seconds before beginning each task and between each sample set. To eliminate any visual and olfactory input, all sensory testing sessions were conducted under red lighting, and participants were asked to wear nose clips during testing. All solutions were served at room temperature, with a three-digit code allocated to each sample.

### Table 2.7

Concentrations (weak, medium, and strong intensity) of prototypical tastants used for determination of suprathreshold taste intensity in Study 1 and Study 2.

<table>
<thead>
<tr>
<th>Taste quality</th>
<th>Stimulus</th>
<th>Concentration (% w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Weak</td>
</tr>
<tr>
<td>Sweet</td>
<td>Sucrose</td>
<td>3.4</td>
</tr>
<tr>
<td>Salty</td>
<td>Sodium chloride</td>
<td>0.6</td>
</tr>
<tr>
<td>Sour</td>
<td>Citric acid</td>
<td>0.02</td>
</tr>
<tr>
<td>Bitter</td>
<td>Caffeine</td>
<td>0.02</td>
</tr>
<tr>
<td>Umami</td>
<td>Monosodium glutamate</td>
<td>0.05</td>
</tr>
</tbody>
</table>

The concentration series were adapted from Webb et al. (70).
Chapter 2 – Materials, Methodology, and Measurements

2.4.10 Five Primary Tastes – Intensity Measurements

2.4.10.1 Participant Training and Standardisation of gLMS with Weight Ratings

The training procedures prior to using the gLMS to rate taste intensity of the five-prototypical taste solutions were similar to the procedures outlined previously in Section 2.4.4.1. Likewise, the standardisation procedures of gLMS usage within participants with weight ratings for the five-prototypical taste solutions were similar to the procedures outlined in Section 2.4.4.2.

2.4.10.2 Five Primary Tastes - Intensity Measurement Procedures

Trays containing three concentrations of prototypical tastant solutions (weak, medium, strong) and a control (blank) solution were prepared to determine perceived ST for the five primary tastes. Each stimulus was presented to participants independently (sets), but in a randomised order. The concentrations for each stimulus ranged from ‘weak’ to ‘strong’ on the gLMS. Prior to tasting the samples, participants were instructed to rinse their mouth with filtered deionised water prior to sampling the set of solutions. The solutions were presented with a randomised three-digit code, and tastings were conducted under red lights. Wearing a nose clip, participants were instructed to place the 15 mL samples into their mouth, hold it in their mouth for 5 seconds, and expectorate. Participants will be required to rate the taste intensity of the samples on the gLMS. Participants were also asked to rinse their mouth for 5 seconds with deionised water between samples.
2.4.11 Hedonic Ratings – Materials and Methods

To measure liking of glucose and maltodextrin solutions in Study 3, three concentrations (weak, medium, and strong) and a control (blank) solution were prepared. These solutions were identical to the concentrations used to assess suprathreshold intensity ratings for glucose and maltodextrin (discussed in Section 2.4.2 and Section 2.4.5). The methods to prepare the solutions were similar to the procedures outlined in Section 2.4.2 and Section 2.4.5.

To assess liking of sweet and complex carbohydrate prototypical foods in Study 3, participants were required to rate their liking of 16 food items (eight sweet tasting and eight complex carbohydrate based foods). The foods included in testing had approximately equivalent fat per 100g. Participants were given a variety of different sweet and complex carbohydrate based foods representing a range of dietary carbohydrate contents per serve (differences in grams of sugar or starch per 100g), approximately equivalent to the concentrations (% w/v) used to measure suprathreshold intensity ratings for glucose and maltodextrin. The foods included in testing can be viewed in Table 2.8 (Appendix L).
Table 2.8 *Sweet and complex carbohydrate based foods used for hedonic ratings in Study 3.*

<table>
<thead>
<tr>
<th>Food</th>
<th>Sugar per 100g</th>
<th>Starch per 100g</th>
<th>Fat per 100g</th>
<th>Amount provided (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red kidney beans, unsalted, canned</td>
<td>0.7</td>
<td>13.1</td>
<td>0.5</td>
<td>20g</td>
</tr>
<tr>
<td>Pasta, elbow, boiled with water, plain</td>
<td>0</td>
<td>28.4</td>
<td>0.3</td>
<td>20g</td>
</tr>
<tr>
<td>Rice, white, boiled with water</td>
<td>0.1</td>
<td>36.0</td>
<td>0</td>
<td>20g</td>
</tr>
<tr>
<td>White bread</td>
<td>3.5</td>
<td>48.0</td>
<td>1.8</td>
<td>20g</td>
</tr>
<tr>
<td>Weet-Bix (Australian breakfast cereal biscuit)</td>
<td>3.3</td>
<td>67.0</td>
<td>1.4</td>
<td>20g</td>
</tr>
<tr>
<td>Pretzel chips, low fat</td>
<td>1.9</td>
<td>72.8</td>
<td>1.6</td>
<td>20g</td>
</tr>
<tr>
<td>Rice cake, thin, plain</td>
<td>0.2</td>
<td>78.0</td>
<td>2.8</td>
<td>20g</td>
</tr>
<tr>
<td>Rice cracker, plain</td>
<td>4.7</td>
<td>81.5</td>
<td>1.1</td>
<td>20g</td>
</tr>
<tr>
<td>Tomato puree (passata), unsalted, canned</td>
<td>8.2</td>
<td>0</td>
<td>1.1</td>
<td>15mL</td>
</tr>
<tr>
<td>Apples, dried</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>20g</td>
</tr>
<tr>
<td>Gelatin dessert, blackcurrant flavoured</td>
<td>19.5</td>
<td>0</td>
<td>0</td>
<td>15g</td>
</tr>
<tr>
<td>Chocolate flavoured syrup, low fat</td>
<td>54.8</td>
<td>0</td>
<td>0.4</td>
<td>15mL</td>
</tr>
<tr>
<td>Strawberry jam</td>
<td>64.0</td>
<td>0</td>
<td>0</td>
<td>15mL</td>
</tr>
<tr>
<td>Raisins</td>
<td>71.4</td>
<td>0</td>
<td>0</td>
<td>20g</td>
</tr>
<tr>
<td>Honey</td>
<td>82.5</td>
<td>0</td>
<td>0</td>
<td>15mL</td>
</tr>
<tr>
<td>Fairy floss (known as cotton candy in the USA, or candy floss in the UK)</td>
<td>97.2</td>
<td>0</td>
<td>0</td>
<td>5g</td>
</tr>
</tbody>
</table>

Food details: Red kidney beans, unsalted, canned (Coles Homebrand, Coles, Australia); Pasta, elbow (Coles Homebrand, Coles, Australia), Rice, white (SunRice, Ricegrowers Limited, Australia); White bread (Coles Homebrand, Coles, Australia); Weet-Bix (Sanitarium Health and Wellbeing Company, Australia); Pretzel chips, low fat (Parker’s, The Smith’s Snackfood Company, Australia); Rice cake, thin, plain (SunRice, Ricegrowers Limited, Australia); Rice cracker, plain (Sakata, The Smith’s Snackfood Company, Australia); Tomato puree (passata), unsalted, canned (Coles Homebrand, Coles, Australia); Apples, dried (Angas Park, Angas Park Fruit Co, Australia); Gelatin dessert, blackcurrant flavoured (Aeroplane Jelly, McCormick & Company, Australia); chocolate flavoured syrup, low fat (Cottee’s, Heinz Foodservice, Australia); Strawberry jam (IXL, SPC Ardmona, Australia); Raisins (Coles Homebrand, Coles, Australia); Honey (Coles Homebrand, Coles, Australia); Fairy floss (The Fairy Floss King, NSW, Australia).
2.4.12 Hedonic Ratings – Measurements

To measure hedonic ratings for sweet and complex carbohydrate solutions, a tray containing three concentrations of glucose/maltodextrin solutions (15 mL each; weak, medium, strong) and a control (blank) solution were prepared. To measure hedonic ratings for sweet and complex carbohydrate prototypical foods, eight small samples (5-20g) per tray were served in a randomised order, and each tray was presented to participants independently. Prior to tasting the samples, participants were instructed to rinse their mouth with filtered deionised water prior to sampling the set of solutions/foods. The solutions were presented with a randomised three-digit code. Participants were also asked to rinse their mouth for 5 seconds with deionised water between samples.

Liking of both solutions and foods was measured using a nine-point hedonic scale ranging from 1 = dislike extremely to 9 = like extremely (Appendix M). All solutions/foods were ingested. Participants were instructed to taste and ingest as much or as little of the sample as desired.

2.4.12.1 Standardisation of Hedonic Scale Usage with Non-Food Items

To control for idiosyncratic scale usage, participants were asked to complete a Likes and Dislikes Questionnaire (Appendix N). (276) Participants were required to rate, on average, how much they liked or disliked a number of food/beverage items and common experiences across ten categories (77 items; i.e. grains/cereals, meat/meat alternatives, fast foods, dairy, fruit and vegetables, snack foods, fats/oils, beverages, oral sensation, non-food) on a nine-point hedonic scale.
Examples of non-food items (4 items) included how much they liked or disliked jumping in a pool on a hot day, or the glare of headlights.

As detailed in Study 3, correlations between the overall mean hedonic ratings for food/beverage items and the overall mean hedonic ratings for non-food items were determined. As individual hedonic ratings for food/beverage items and non-food items were assumed to be unrelated, if a significant correlation was observed, it indicated that the hedonic scale ratings were subject to differences in individual scale-use and required standardisation across participants. To determine a personal standardisation factor, a similar calculation method with gLMS standardisation was used (i.e. the grand mean for non-food items across all participants was divided by each participant’s average hedonic ratings for non-food items). Each individual’s hedonic ratings were multiplied by his or her personal standardisation factor for scale-use bias.

2.5 Body Composition

All participants were asked to remove shoes and heavy clothing to ensure accurate measurements. All body composition measurements were measured first thing during the initial visit after a 1-hour fast (food only). For Study 1 and Study 2, body composition measurements (weight and waist circumference only) were repeated first thing during the final visit after a 1-hour fast (food only). Participants’ body weight was measured to the nearest 0.1 kg using a segmental body composition analyser (TBF-300A) (Tanita Corporation, Tokyo, Japan). Participants’ height was measured to the nearest 0.1 cm using a portable stadiometer (Seca213) (Seca, Hamburg, Germany). All measurements were repeated twice to ensure accuracy. Averages of height and weight measurements
were used to calculate BMI (weight in kg/m²) and determine weight status (*i.e.* normal weight or overweight/obese). Weight status was defined under World Health Organisation BMI classification. (277) Using methods outlined by the Australian Heart Foundation, (278) waist circumference was also measured. Waist circumference was measured twice to the nearest 0.1 cm using an ergonomic circumference measurement tape (Seca201) (Seca, Hamburg, Germany). An average of waist circumference measurements was calculated and used for analysis. Waist circumference cut-off points [*i.e.* lower risk of metabolic complications or increased risk of metabolic complications (> 94cm males, > 80cm females)] were defined under the World Health Organisation recommended cut-off points. (279)

### 2.6 Dietary Intake

#### 2.6.1 Dietary Questionnaire for Epidemiological Studies Version 2

The Dietary Questionnaire for Epidemiology Studies Version 2 (DQESV2), (280) a validated version of the Food Frequency Questionnaire (FFQ) (281, 282) developed by Cancer Council Victoria was used to measure each participant’s habitual pattern of food intake in **Study 1** and **Study 2** (*Appendix O*). Participants were required to indicate, on average, how many times in the previous year they consumed a number of food and beverage items (74 items) across four categories (*i.e.* 1) cereal foods; sweets and snacks; 2) dairy products, meats and fish; 3) fruit; and 4) vegetables) with 10 frequency response options ranging from ‘never’ to ‘3 or more times per day’. They were also required to indicate the portion size that they normally consumed. Participants were asked to complete the FFQ within a month from their first visit. Using software based on the Australian nutrient
composition database NUTTAB95, (283) analysis was carried out by the Cancer Council Victoria Australia to assess daily energy and macronutrient intakes (blinded from sensory testing results and aims of the studies).

2.6.2 Deakin Food Frequency Questionnaire

For Study 3, an adapted version of the 1995 Australian National Nutrition Survey FFQ (284) was used to measure each participant’s habitual pattern of food intake (Appendix P). Participants were required to indicate, on average, how many times in the previous month they consumed a number of food and beverages and vitamin and mineral supplements (118 items; bread and cereal foods, dairy foods, meat, fish, eggs, sweets, baked goods, and snacks, dressings, non-dairy beverages, vegetables, fruits). Participants were instructed to select the most appropriate answer on a nine-point scale with response options ranging from ‘never or less than once per month’ to ‘6 or more times per day’. In order to conduct the appropriate statistical analyses, response options for consumption variables were collapsed. (285) For example, the white bread category was recoded from the original nine response options, down to three response options (Appendix Q). A different questionnaire was used for Study 3 this questionnaire can be used as an online survey tool. This would minimise risk of participants losing the questionnaires.

2.6.3 Diet Diary

In addition to the DQESV2 (Cancer Council Victoria FFQ), participants were asked to complete a 4-day diet diary (3 weekdays, 1 weekend day within a 7-day period; Appendix S) in which they recorded all of the foods and beverages
they consumed within a month from their first visit in Study 2. Participants were asked to, where possible, measure their foods using measurement cups, spoons or common serving sizes (e.g. one large egg) or to weigh their foods using kitchen scales at home. They were also asked to be as specific as possible, including reporting the type (e.g. skim milk or full fat milk) and brand of food consumed, the cooking methods used (e.g. fried, baked, or steamed) or whether fat was added when cooking (e.g. food cooked in butter or cooking oil). If the food consumed was from a recipe, the participants were asked to include the recipe with the record and to state how much of it they consumed (e.g. a quarter of a recipe). Participants were also given an example of a 1-day diet diary record as a guide to complete the diet diaries. Diet diaries were analysed using FoodWorks 8 (Xyris Software, Highgate Hill, Queensland, Australia). Mean energy intake (kilojoules; kJ) and macronutrient distribution (% energy from fat, protein, and carbohydrate), and the type of carbohydrate (% energy from starch, sugar, fibre) were quantified using the Australian nutrient composition database AUSNUT2011-13 (286) and compliance checked.

2.6.4 Non-Nutritive Sweetener Consumption

In Study 1, participants were asked how often they consumed foods and/or beverages sweetened with NNS (artificial sweeteners, natural NNS) by selecting an appropriate response category from a list of ‘more than once a day’, ‘once per day’, ‘three to six times per week’, ‘once or twice per week’, ‘one to three times per month’, ‘once per month or less’, or ‘never’ (Appendix S). (287) Participants were given examples of commercial products that were sweetened with NNS (both artificial and natural NNS sweetened) such as low-energy carbonated drinks,
confectionary and dairy products. Participants were also instructed to include NNS consumed with tea and coffee.

2.7 Three-Factor Eating Questionnaire

A dietary restraint score was measured according to factor one of the Three-Factor Eating Questionnaire (1) in Study 3(b). Participants answered 51 questions relating to their eating habits and feelings towards eating. For example, one item was ‘When I have eaten my quota of calories, I am usually good about not eating any more’ (Appendix T). Based on their answer to each question, participants received either a 0 or 1 point for each of the questions. Participants identified as restrained eaters (defined by a score of > 11 on factor one of the Three-Factor Eating Questionnaire) were removed from the analyses (1) in Study 3(b).

2.8 Satiation Measures - Preload and Ad Libitum Intake of Milkshakes, Drinking Rate, Appetite, and Hedonic Ratings

2.8.1 Satiation Measures - Materials

The sweet (glucose) and complex carbohydrate (maltodextrin) milkshakes (per 100g) used to assess the effect of satiation in Study 3(b) consisted of: 8.8% glucose/maltodextrin (The Melbourne Food Depot, Melbourne, Australia; Star-Dri 5, Tate & Lyle Ingredients Americas, USA), 63.7% long-life skim milk (99.9% fat free; Devondale Murray Goulburn, Melbourne, Australia), 26.5% light thickened cream (~18% fat; Bulla, Derrimut, Australia), and 1.0% imitation vanilla essence (Queen Fine Foods, Alderley, Australia). The nutrient compositions of the milkshakes were calculated using Foodworks8 (Xyris Software) (Table 2.9).
milkshakes were mixed until no lumps were visible using an immersion (stick) blender for 15 seconds (per 100g) at 10,000 rpm (KitchenAid KHB2569 Hand Blender, Whirlpool Corporation, Michigan, USA). All milkshakes were prepared fresh on the day of testing and stored refrigerated (± 3°C) using plastic food storage containers.

<table>
<thead>
<tr>
<th>Table 2.9</th>
<th>Nutrient composition of sweet and complex carbohydrate milkshakes containing different amounts of glucose and maltodextrin (Study 3(b)).</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sweet Milkshake</td>
</tr>
<tr>
<td></td>
<td>Per 100g</td>
</tr>
<tr>
<td>Energy, kJ</td>
<td>454.3</td>
</tr>
<tr>
<td>Carbohydrate, g</td>
<td>13.2</td>
</tr>
<tr>
<td>Sugars, g</td>
<td>12.8</td>
</tr>
<tr>
<td>Starch, g</td>
<td>0.4</td>
</tr>
<tr>
<td>Protein, g</td>
<td>2.8</td>
</tr>
<tr>
<td>Fat, g</td>
<td>5.3</td>
</tr>
</tbody>
</table>

The nutrient composition of the milkshakes (8.8% glucose/maltodextrin, 63.7% long-life skim milk, 26.5% light thickened cream, and 1% imitation vanilla essence) per 100g was calculated using Foodworks8 (Xyris Software).

2.8.2 Satiation Measures – Methods and Measurements

A modified procedure outlined by Rolls and McDermott (1991) was used to assess the satiation effect of sweet and complex carbohydrate milkshakes in Study 3(b). Participants were first served a cup containing 200g of milkshake (sweet: 908.6kJ, complex carbohydrate: 881.4kJ), and were instructed to finish the whole cup of milkshake within a minute (maximum time). Two minutes after consumption of the preload milkshake, participants were presented with another serving of the same milkshake (600g; sweet: 2725.8kJ, complex carbohydrate: 2644.2kJ). For the 600g milkshake, participants were told to drink until they were comfortably full (maximum time: 5 minutes). The serving sizes for preload (200g) and ad libitum (600g) milkshakes were derived through previously published
finding by Rolls and McDermott (288) using young adult samples. In that study, (288) a fixed volume of yogurt (300g) was given to participants as a preload as it was found to be the average amount of yogurt consumed by participants. However, as the participants in Study 3(b) were mainly young female adults, we chose to use a smaller portion (200g) as the serving size for preloads to be sure that participants were given the opportunity to drink until satiated during the *ad libitum* experiment.

A concentration of 8.8% (per 100g of complex carbohydrate milkshake) of maltodextrin was used based on previous published findings of this concentration having a perceptually distinctive taste sensation without perceivable viscosity. (18, 289) A concentration of 8.8% (per 100g of sweet milkshake) of glucose was used for sweet milkshakes. During the milkshake experiment, participants were not allowed to drink any water until after the experiment was over. The *ad libitum* milkshake intake was calculated as the difference in the weight of the cup of milkshake before and after consumption. The milkshake intake in grams was used to determine the energy intake in kilojoules. Drinking rate (g/sec or kJ/sec) was calculated by dividing the *ad libitum* milkshake intake in grams or kilojoules by the total drinking duration (sec). During the milkshake experiment, participants were asked to start drinking the milkshake as soon as they were instructed to start, and to raise their hands quietly to inform the researcher in the room as soon as they had finished. The researcher, using a stopwatch, measured the total duration (sec) used to drink the *ad libitum* milkshake.

Prior to consuming the preload and *ad libitum* milkshakes, participants completed several questions relating to appetite and hedonic ratings (*Appendix U*). (213, 256, 290, 291) When the milkshakes were served, participants were instructed to drink a sip of their milkshake and to rate their liking of it on a nine-point hedonic...
scale. Participants were also instructed to rate their feelings of hunger, fullness, and prospective consumption prior to consumption of both milkshakes (preload and *ad libitum*) on a 100 mm visual analogue scale (VAS) anchored at each end with descriptors (e.g. ‘not hungry at all’ at one end and ‘very hungry’ at the other).
Chapter Three: Study 1(a) Psychophysical Evaluation of Sweetness Functions across Multiple Sweeteners

This study has been published in Chemical Senses (2016): bjw109 as ‘Psychophysical Evaluation of Sweetness Function across Multiple Sweeteners’

3.1 Introduction

A range of sweetness intensities can be experienced when sweet-tasting compounds activate sweet taste receptor cells in areas of the tongue, soft palate, and oropharyngeal region of the oral cavity. (292) For instance, when 1 mM sucrose is dissolved in water, an individual may find it challenging to differentiate the sucrose-containing solution from plain water. However, as the concentration of sucrose is increased, differentiation becomes possible. (54, 69, 70) The lowest concentration level at which a difference can be detected is termed the sucrose detection threshold. At this concentration level the individual cannot accurately identify the sucrose solution as sweet, and only when the concentration of sucrose is further increased does the sweet taste quality become apparent. (293) The lowest concentration at which this occurs is termed the sucrose recognition threshold. (54, 72, 293) As sucrose is progressively added beyond this point the perceived sweetness will range from just perceivable to strong, until it reaches the individual’s terminal threshold for sucrose, beyond which any increase in concentration no longer causes consequential increase in perceived sweetness intensity. (54, 73, 74) Perceived sweetness above the
recognition threshold is defined as the suprathreshold intensity perception range. (69, 74)

Theoretically, it seems reasonable to expect that an individual’s sweetness detection threshold, recognition threshold, and suprathreshold intensity perception might be interrelated. (54, 69, 74-76) An example of this hypothetical model was observed from a bitter compound, 6-n-Propylthiouracil (PROP). For example, an individual who is able to detect and/or recognise bitterness from PROP at a lower concentration level may, when tasting a concentrated PROP solution, be more likely to experience greater bitterness intensity than another individual with a higher bitterness detection threshold for PROP (i.e. strong negative correlation between detection threshold and suprathreshold intensity for PROP). (54, 77) This, however, has not been confirmed for sweet compounds. (70, 78)

Previous human psychophysical studies have consistently found large individual variation in the capability to perceive sweet taste from sucrose (70, 153-158, 294) and/or a range of sweeteners. (159-161) Such individual variation may be due to differences in human physiology [e.g. variation in the human TAS1R2 gene (295)] or cognitive functioning when perceiving a sweet stimulus. (70) Most human psychophysical studies investigating sweet taste, however, have employed only one measure of taste function to measure sweet taste. As each measure of sweet taste function represents a different dimension of the sense of taste, there is currently no single method to measure taste function in totality. (70) Although the transduction mechanisms of sweet taste (111) and the perceptual relationships between caloric sweeteners (296, 297) and non-nutritive sweeteners (298) have been reported, collecting a range of psychophysical measures across multiple sweeteners within a
single group of individual allows direct comparison that cannot be made across prior studies.
3.2 Aims and Hypotheses

3.2.1 Aims

The aim was to investigate the three main measures of sweet taste function - detection threshold, recognition threshold and suprathreshold intensity - across a range of caloric and non-nutritive sweeteners.

3.2.2 Hypotheses

- There will be significant individual variance in sweet taste sensitivities for detection and recognition thresholds. There will also be significant individual variance in perceived sweetness intensity. Participants will be able to be classified into more sensitive/experienced high intensity, normal sensitive/moderate intensity, and less sensitive/low intensity groups according to the sweetness of various sweeteners, and sweet taste measures.

3.3 Subjects, Materials, and Methods

3.3.1 Subjects

Sixty participants (28 male), 18-52 years of age (mean age in years = 26, SD = ±7.8), were recruited from locations adjacent to the Deakin University, Melbourne campus, Australia. Potential participants were excluded if they: (1) smoked; (2) were pregnant or lactating; (3) were taking any prescription medication that may interfere with their ability to taste; or (4) had a history of food allergies that may interfere with the study. Participants were asked to refrain from eating, drinking (except room temperature water), brushing their teeth, and chewing gum for one hour prior to
testing. All participants gave written informed consent and were compensated for their participation. This study was approved by the institutional review board regulations of Deakin University (DUREC 2013-156). The experimental protocol was also registered under the Australian New Zealand Clinical Trials Registry (ACTRN12613000701729), www.anzctr.org.au. This study also complies with the Declaration of Helsinki for Medical Research involving Human Subjects.

3.3.2 Study Design

This study comprised three measures of taste perception routinely used in chemosensory research: (1) detection threshold (DT), (2) recognition threshold (RT), and (3) suprathreshold intensity. These measures were determined for all participants for each of six sweeteners and prototypical stimuli for salty, sour, bitter, and umami during a total of 16 sessions (two sessions per day separated by a minimum of one hour for eight non-consecutive days). All measurements were collected in duplicate. If there were more than three concentration steps between the duplicate measures, participants attended another session to complete the assessment. DT, RT, and suprathreshold intensity tasks were conducted in computerised, partitioned sensory booths in the Centre for Advanced Sensory Science using Compusense Five Software Version 5.2 (Compusense Inc., Ontario, Canada). Filtered deionised water was used as an oral rinsing agent. Participants were instructed to rinse their mouths with filtered deionised water for five seconds before beginning each task and between each sample set. To eliminate any visual and olfactory input, all testing sessions were conducted under red lighting, and participants were asked to wear nose clips during testing. All solutions were served at room temperature, with a three-digit code allocated to each sample. Participants in the present study were part of a larger study focusing on the
Chapter 3 – Study 1(a) Psychophysical Evaluation of Sweetness Functions across Multiple Sweeteners

association between sweet taste function, body composition, and dietary intake (Chapter 4). (299)

3.3.3 Participant Training

Prior to using the general Labeled Magnitude Scale (gLMS) to rate taste intensity, participants were trained using the standard protocol outlined by Green et al. (266, 267) except the top of the scale was described as the strongest imaginable sensation of any kind (Appendix G, described in Chapter 2). (75) The 100-point scale comprised the following adjectives: ‘no sensation’ = 0, ‘barely detectable’ = 1.5, ‘weak’ = 6, ‘moderate’ = 17, ‘strong’ = 35, ‘very strong’ = 52, and ‘strongest imaginable’ = 100. (75) Only scales with adjectives were presented to participants (no equivalent numbers, although numerical data were extracted from the scale for data analysis). (70) During the training session, participants were asked to rate the intensity of the perceived sensation relative to a remembered or imagined sensation. Participants were required to rate a list of seven remembered or imagined sensations, such as the warmth of lukewarm water, the pain from biting of the tongue, and the sweetness of fairy floss (known as cotton candy in the USA, or candy floss in the UK) (Appendix H).

3.3.4 Stimuli

Prototypical stimuli (sucrose, sodium chloride, citric acid, caffeine, and monosodium glutamate) were used to investigate taste function for the five basic tastes (for details of stimuli see Table 3.1; described in Chapter 2). Both caloric (glucose monohydrate, fructose, sucrose, erythritol) and non-nutritive sweeteners (NNS)
(sucralose, Rebaudioside A) were used to investigate sweet taste (for details of stimuli see Table 3.2). On the morning of testing, solutions were prepared with filtered deionised water (Cuno Filter Systems FS117S, Meriden, CT, USA) and stored in glass beakers at room temperature (20 ± 1 °C).

3.3.5 Detection and Recognition Threshold Determination for the Five Primary Tastes

As described in Chapter 2, DT and RT were determined using the procedure outlined in the International Standards Organisation (ISO) Method of Investigating Sensitivity of Taste. (265) Table 3.1 gives the nine concentrations used for each taste quality (the ninth concentration being presented only when participants were unable to recognise the taste quality in the previous eight). (265) The eight samples from each taste quality were served in ascending concentration (15 mL per sample, in accordance with the standard ISO method), and each taste quality was presented to participants independently. Participants were unaware of the presentation order but were informed of the possible taste qualities. Participants were instructed to taste each sample for five seconds then expectorate and record whether: there was an absence of taste (water-like); a taste was identified but not recognised; or a taste quality was perceived. (265) DT was defined as the concentration at which the participants selected the ‘taste identified, but unknown taste quality’ response. (265) RT was defined as the concentration at which they were able to recognise the correct taste quality twice consecutively. (70)
Table 3.1 *Stimulus concentrations used for prototypical threshold testing.*

<table>
<thead>
<tr>
<th>Taste quality</th>
<th>Stimulus</th>
<th>Concentrations (% w/v)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweet</td>
<td>Sucrose</td>
<td>0.03</td>
<td>0.06</td>
<td>0.09</td>
<td>0.1</td>
<td>0.2</td>
<td>0.4</td>
<td>0.7</td>
<td>1.2</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>Salty</td>
<td>Sodium chloride</td>
<td>0.01</td>
<td>0.02</td>
<td>0.03</td>
<td>0.05</td>
<td>0.07</td>
<td>0.10</td>
<td>0.14</td>
<td>0.20</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Sour</td>
<td>Citric acid</td>
<td>0.013</td>
<td>0.016</td>
<td>0.020</td>
<td>0.025</td>
<td>0.031</td>
<td>0.038</td>
<td>0.048</td>
<td>0.06</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Bitter</td>
<td>Caffeine</td>
<td>0.006</td>
<td>0.007</td>
<td>0.009</td>
<td>0.011</td>
<td>0.014</td>
<td>0.017</td>
<td>0.022</td>
<td>0.027</td>
<td>0.045</td>
<td></td>
</tr>
<tr>
<td>Umami</td>
<td>Monosodium glutamate</td>
<td>0.008</td>
<td>0.012</td>
<td>0.017</td>
<td>0.024</td>
<td>0.034</td>
<td>0.049</td>
<td>0.070</td>
<td>0.10</td>
<td>0.17</td>
<td></td>
</tr>
</tbody>
</table>

The concentration series were adapted from ISO3972. (261) Reference chemical details: sucrose (CSR, Yarraville, Australia); sodium chloride (Saxa, Premier Foods Inc, Seven Hills, Australia); caffeine (Sigma Aldrich, Steinham, Germany); citric acid (Ward McKenzie Private Limited, Altona, Australia); and monosodium glutamate (Ajinomoto Cooperation, Tokyo, Japan).
<table>
<thead>
<tr>
<th>Sweetener</th>
<th>Concentration (% w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Glucose Monohydrate</td>
<td>0.02</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.01</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.01</td>
</tr>
<tr>
<td>Sucralose (x10^{-3})</td>
<td>0.02</td>
</tr>
<tr>
<td>Rebaudioside A (x10^{-3})</td>
<td>0.03</td>
</tr>
<tr>
<td>Erythritol</td>
<td>0.02</td>
</tr>
</tbody>
</table>

The concentration series for sucrose was adapted from ISO3972. (261) The concentration series for glucose monohydrate, fructose, sucralose, Rebaudioside A, and erythritol were prepared in successive 0.25 log dilution steps. Reference chemical details: glucose monohydrate (The Melbourne Food Depot, Melbourne, Australia); fructose (The Melbourne Food Depot, Melbourne, Australia); sucrose (CSR, Yarraville, Australia); sucralose (The Melbourne Food Depot, Melbourne, Australia); Rebaudioside A (AuSweet, Melbourne, Australia); and erythritol (AuSweet, Melbourne, Australia).
3.3.6 Detection and Recognition Threshold Determination for Sweet Taste

Detailed in Table 3.2 are the concentration ranges used to assess DT and RT for sweet taste. The concentration series for sucrose was adapted from ISO3972; (261) concentrations for the remaining sweeteners were prepared with successive 0.25 log dilution steps. Initial starting concentrations were determined through informal bench-top testing, based on modified findings of matching sweetness intensity ratios published by Keast et al. (262). DTs for each of the sweeteners were determined using ascending forced choice triangle methodology, (263, 264) in which the participants were provided with three 25 mL samples, two of which were controls (filtered deionised water) and one containing sweetener, in ascending order from the lowest to the highest concentration. DT was defined as the concentration of sweetener required for a participant to correctly identify the sweetened sample as the odd one out in three consecutive sample sets at one concentration level. (263) The RTs for each of the sweeteners were measured using a whole-mouth, sip-and-spit procedure, as described in Chapter 2. (76)

3.3.7 Suprathreshold Intensity Ratings for the Five Prototypical Tastes and Sweeteners

Three concentrations (weak, medium, and strong) and a blank (control) sample were prepared to determine perceived suprathreshold intensity for each prototypical tastant (Table 3.3) and sweetener (Table 3.4), as described in Chapter 2. These concentrations were derived through informal bench-top testing (ascending taste intensity) and were similar to the concentrations outlined by Webb et al. (70). The
concentrations for each prototypical stimulus ranged from ‘weak’ to ‘strong’ on the gLMS. These samples were presented to participants in randomised order.

Table 3.3 *Concentrations (weak, medium, and strong intensity) of prototypical tastants used for determination of suprathreshold taste intensity.*

<table>
<thead>
<tr>
<th>Taste quality</th>
<th>Stimulus</th>
<th>Concentration (% w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Weak</td>
</tr>
<tr>
<td>Sweet</td>
<td>Sucrose</td>
<td>3.4</td>
</tr>
<tr>
<td>Salty</td>
<td>Sodium chloride</td>
<td>0.6</td>
</tr>
<tr>
<td>Sour</td>
<td>Citric acid</td>
<td>0.02</td>
</tr>
<tr>
<td>Bitter</td>
<td>Caffeine</td>
<td>0.02</td>
</tr>
<tr>
<td>Umami</td>
<td>Monosodium glutamate</td>
<td>0.05</td>
</tr>
</tbody>
</table>

The concentration series were adapted from Webb et al. (70).

Table 3.4 *Concentrations (weak, medium, and strong intensity) of sweeteners used for determination of suprathreshold taste intensity.*

<table>
<thead>
<tr>
<th>Sweetener</th>
<th>Concentration (% w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weak</td>
</tr>
<tr>
<td>Glucose monohydrate</td>
<td>5.3</td>
</tr>
<tr>
<td>Fructose</td>
<td>2.9</td>
</tr>
<tr>
<td>Sucrose</td>
<td>3.4</td>
</tr>
<tr>
<td>Sucralose (x10^{-3})</td>
<td>5.7</td>
</tr>
<tr>
<td>Rebaudioside A (x10^{-3})</td>
<td>8.6</td>
</tr>
<tr>
<td>Erythritol</td>
<td>5.7</td>
</tr>
</tbody>
</table>
Chapter 3 – Study 1(a) Psychophysical Evaluation of Sweetness Functions across Multiple Sweeteners

3.3.8 Standardisation of gLMS Usage with Weight Ratings

As described in Chapter 2, to standardise gLMS usage within participants, a modified version of Delwiche et al. (268) was adapted for this study. To control for idiosyncratic scale usage, participants were asked to rate the heaviness of six, visually identical weights (opaque bottles filled with sand and stone and completely wrapped in aluminium foil; weights of 53, 251, 499, 724, 897, and 1127g) (Appendix I). Participants were asked to hold out their non-dominant hand palm up, while the experimenter placed the weighted bottle on the palm of the hand. Participants were instructed to rate the heaviness of each weight using the gLMS.

There was a significant correlation between the overall mean prototypical ratings and overall mean heaviness ratings ($r = 0.28$, $P < 0.05$). Assuming that the intensity ratings of prototypical tastants and the heaviness of the bottles were unrelated, the significant correlation indicates that the gLMS ratings were subject to differences in individual scale-use and thus require standardisation across participants. (54, 70, 268) To determine a personal standardisation factor, the grand mean for heaviness across weight levels and participants was divided by each participant’s average intensity for heaviness. (54) Each individual’s prototypical taste intensity and sweetness intensity ratings were multiplied by his or her personal standardisation factor for scale-use bias. (54, 268)

3.3.9 Statistical Analysis

Statistical analysis was performed using IBM SPSS statistical software version 23.0 (SPSS, Chicago, IL, USA). Data are presented as means with standard errors of mean (SEM). For suprathreshold intensity ratings, the geometric mean score of the
three ratings (weak, medium, and strong) was calculated. Spearman’s rank correlation coefficient was calculated between distinct measures of taste function and to minimise risk of type 1 error.

As noted earlier, if the measures of taste function are interrelated, then participants who are less sensitive to the sweet compounds tested should have higher detection and recognition thresholds and lower sweetness intensity ratings than the more sensitive participants (who should have lower detection and recognition thresholds and higher sweetness intensity ratings). That is, the correlation between the threshold measures (DT and RT) and suprathreshold intensity should be negative. In order to simplify the data presentation, negative $r$-values were converted to positive and vice versa. The criterion for statistical significance was set at $P < 0.05$. 
3.4 Results

3.4.1 Detection and Recognition Thresholds of Sweeteners

For all sweeteners, the test-retest correlation reached significance for both detection (ICC = 0.876-0.963), recognition (ICC = 0.869-0.943), and suprathreshold intensity (ICC = 0.678-0.969), all *P* < 0.05.

Mean (±SEM) DT and RT values for the sweeteners are presented in Table 3.5. There was large individual variation among the participants; for example DT for glucose ranged from 0.02 % w/v to 1.8 % w/v, while the RT for glucose ranged from 0.05 % w/v to 2.9 % w/v (Figure 3.1a).

<table>
<thead>
<tr>
<th>Detection Threshold</th>
<th>Recognition Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean ± SEM</strong></td>
<td><strong>Range</strong></td>
</tr>
<tr>
<td>Glucose</td>
<td>0.34 ± 0.04</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.20 ± 0.03</td>
</tr>
<tr>
<td>Sucralose (x10^-3)</td>
<td>0.52 ± 0.07</td>
</tr>
<tr>
<td>Rebaudioside A (x10^-3)</td>
<td>0.60 ± 0.06</td>
</tr>
<tr>
<td>Erythritol</td>
<td>0.28 ± 0.04</td>
</tr>
</tbody>
</table>

The DTs of caloric sweeteners (glucose, fructose, sucrose, and erythritol) were strongly correlated with one another (*r* = 0.82–0.90, *P* < 0.001; Figure 3.2). However, there were only moderate correlations between the DT of the caloric sweeteners and NNS (*r* = 0.34-0.48, *P* < 0.001), except between the DT of two of the caloric sweeteners (glucose, fructose) and NNS (sucralose) where no correlations were observed (*P* > 0.05).
Figure 3.1 Frequency distributions of detection and recognition thresholds for: a) glucose, b) fructose, c) sucrose, d) sucralose, e) Rebaudioside A, and f) erythritol.
Figure 3.2 Scatter plot matrix and spearman rank correlations of detection thresholds for sweeteners evaluated. **P < 0.01.
Similarly, there were strong correlations between participants’ RTs for all caloric sweeteners \((r = 0.62–0.84, P < 0.001; \text{Figure 3.3})\). The RT for sucralose was moderately correlated with sucrose \((r = 0.32, P < 0.05)\), but not with other caloric sweeteners \((all \ P > 0.05)\). RT for Rebaudioside A was moderately correlated with glucose, fructose, and sucrose \((r = 0.26-0.30, P < 0.05)\), but not with erythritol \((P > 0.05)\). Moreover, the RTs for the NNS, sucralose and Rebaudioside A, were moderately correlated with each other \((r = 0.39, P < 0.01)\).

### 3.4.2 Suprathreshold Intensities for Sweeteners

\textbf{Figure 3.4} shows the psychophysical functions for all sweeteners. As expected there were monotonic increases in perceived intensity as the concentration of the stimuli was increased. Spearman’s rank correlation revealed a significant relationship between the sweetness ratings on a sweetener’s psychophysical function: (glucose \(r = 0.79-0.93, P < 0.001\); fructose \(r = 0.67-0.89, P < 0.001\); sucrose \(r = 0.73-0.94, P < 0.001\); erythritol; \(r = 0.81-0.91, P < 0.001\); Rebaudioside A; \(r = 0.55-0.83, P < 0.001\); sucralose; \(r = 0.64-0.90, P < 0.001\)). Analysis of variance revealed significant differences between all incremental steps on the psychophysical functions \((P < 0.05)\). This indicates that when a participant is given increasing concentration of a sweetener (above the RT), there is an ordinal increase in sweetness intensity relative to intensity ratings across all participants. For each participant, there were strong correlations between the sweetness intensity of all sweeteners \((r = 0.70–0.95, P < 0.001; \text{Figure 3.5})\).
Chapter 3 – Study 1(a) Psychophysical Evaluation of Sweetness Functions across Multiple Sweeteners

Figure 3.3 Scatter plot matrix and spearman rank correlations of recognition thresholds for sweeteners evaluated. * $P < 0.05$, ** $P < 0.01$. 
Figure 3.4 Mean psychophysical functions for suprathreshold taste intensity together with examples of a participant who experienced high intensity and low intensity: a) glucose, b) fructose, c) sucrose, d) sucralose, e) Rebaudioside A and f) erythritol.
Figure 3.5 Scatter plot matrix and spearman rank correlations of sweetness intensity ratings for sweeteners evaluated. 

*P < 0.05, **P < 0.01
3.4.3 Relationships between Sweet Taste Measures

Strong correlations between DT and RT were observed for all sweeteners ($r = 0.58–0.68$, $P < 0.001$). However, no significant correlation was observed between sweetness intensity ratings and DT or RT for any of the sweeteners tested (all $P$ values $> 0.05$).

3.4.4 Detection and Recognition Thresholds of Prototypical Tastants

DT and RT of the five-prototypical tastes are presented in Table 3.6. DT of sweet, salty, and umami were strongly correlated with each other ($r = 0.56-0.89$, all $P$ values $< 0.001$). However, DT of sour and bitter were not correlated with the other taste qualities ($P$ values $> 0.05$). RT of sour, sweet, umami, and salty were positively correlated with each other ($r = 0.34-0.79$, $P < 0.05$). There were also strong correlations between sucrose DT and RT as obtained by the ascending forced choice triangle method and the ISO method ($r = 0.64-0.66$, $P < 0.001$).
Table 3.6 Detection and recognition thresholds (% w/v) for prototypical tastants, including mean, standard error (SEM), and range.

<table>
<thead>
<tr>
<th>Taste quality</th>
<th>Stimulus</th>
<th>Detection threshold</th>
<th>Recognition threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SEM</td>
<td>Range</td>
</tr>
<tr>
<td>Sweet</td>
<td>Sucrose</td>
<td>0.50 ± 0.02</td>
<td>0.03 – 0.70</td>
</tr>
<tr>
<td>Salty</td>
<td>Sodium chloride</td>
<td>0.03 ± 0.002</td>
<td>0.01 – 0.07</td>
</tr>
<tr>
<td>Sour</td>
<td>Citric acid</td>
<td>0.015 ± 0.002</td>
<td>0.013 – 0.022</td>
</tr>
<tr>
<td>Bitter</td>
<td>Caffeine</td>
<td>0.008 ± 0.0004</td>
<td>0.006 – 0.017</td>
</tr>
<tr>
<td>Umami</td>
<td>Monosodium glutamate</td>
<td>0.055 ± 0.006</td>
<td>0.008 – 0.17</td>
</tr>
</tbody>
</table>
3.4.5 Suprathreshold Intensities of Prototypical Tastants and Relationship with Detection and Recognition Thresholds

As expected, there were monotonic increases in perceived intensity as the concentrations of stimuli were increased (Table 3.7). No correlations were observed between suprathreshold intensities and DT of sweet, salty, sour, and umami (all \( P \) values > 0.05). A correlation was observed for bitter \((r = 0.52, P < 0.01)\). Similarly, no correlations were observed between suprathreshold intensities and RT of sweet, sour, and umami (all \( P \) values > 0.05). Correlations were observed for bitter and salty \((r = 0.31-0.48, P < 0.05)\).

Table 3.7 Suprathreshold intensity ratings for prototypical tastants on gLMS, given by mean and standard error (SEM).

<table>
<thead>
<tr>
<th>Taste quality</th>
<th>Stimulus</th>
<th>Concentration (% w/v)</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweet</td>
<td>Sucrose</td>
<td>3.4</td>
<td>10.1 ± 1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.9</td>
<td>20.2 ± 1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13.7</td>
<td>25.4 ± 1.6</td>
</tr>
<tr>
<td>Salty</td>
<td>Sodium chloride</td>
<td>0.6</td>
<td>17.6 ± 1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.2</td>
<td>20.2 ± 1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.3</td>
<td>27.3 ± 1.9</td>
</tr>
<tr>
<td>Sour</td>
<td>Citric acid</td>
<td>0.02</td>
<td>11.2 ± 1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.04</td>
<td>19.6 ± 1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.08</td>
<td>26.7 ± 1.9</td>
</tr>
<tr>
<td>Bitter</td>
<td>Caffeine</td>
<td>0.02</td>
<td>9.2 ± 1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.06</td>
<td>19.1 ± 1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.13</td>
<td>25.5 ± 1.7</td>
</tr>
<tr>
<td>Umami</td>
<td>Monosodium glutamate</td>
<td>0.05</td>
<td>12.8 ± 1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.10</td>
<td>14.0 ± 1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.20</td>
<td>16.3 ± 1.5</td>
</tr>
</tbody>
</table>
3.4.6 Relationships between Sweet Taste Function and Prototypical Taste Function

Participants were stratified into tertile groups according to the sweeteners tested and all sweet taste measures. We observed that those who were able to detect sucrose in water at lower concentrations (lower tertile; \( n = 8 \)) were also more sensitive to all of the sweeteners tested. Interestingly, we also observed that six participants were more sensitive only towards caloric sweeteners but not to NNS (lower tertile). Similarly for RT, those who were able to recognise sweetness from sucrose at a lower concentration (lower tertile; \( n = 4 \)) were also able to recognise sweetness from all six sweeteners tested at lower concentration levels. When separated according to caloric sweeteners and NNS, four participants were more sensitive (lower tertile) to caloric sweeteners but not to NNS. In contrast, three participants were more sensitive (lower tertile) to NNS but not to caloric sweeteners. For sweetness intensity ratings, we observed that some participants experienced high intensity (higher tertile; \( n = 9 \)) or low intensity (lower tertile; \( n = 14 \)) for all sweeteners measured. One participant was more sensitive or experienced high intensity to all sweeteners and across all sweet taste measures.

When participants were further stratified into tertile groups according to the prototypical tastes and all taste function measures, we observed that some were more sensitive or less sensitive towards all five prototypical tastes within a single taste measure [DTs either low (more sensitive; \( n = 4 \)) or high (less sensitive; \( n = 1 \)); RTs either low (more sensitive; \( n = 5 \)) or high (less sensitive; \( n = 4 \)); sweetness intensities either low (less sensitive; \( n = 5 \)) or high (more sensitive; \( n = 4 \)]. These findings refute the notion of generalised hypergeusia, (70, 300) and suggest there is a great deal of inter-individual variation both across and within measures of a quality. Of particular
Chapter 3 – Study 1(a) Psychophysical Evaluation of Sweetness Functions across Multiple Sweeteners

note, no participant was more sensitive/experienced high intensity or less sensitive/experienced low intensity to all taste qualities across all taste measures.

3.5 Discussion

Our data suggest that threshold sensitivity (both DT and RT) to the sweetness of caloric sweeteners (glucose, fructose, and sucrose) does not necessarily imply threshold sensitivity to NNS (sucralose and Rebaudioside A). On the contrary, the present data are more supportive of the hypothesis that caloric sweeteners and NNS access at least partially independent peripheral physiology responsible for DT and RT measures. (111)

The prevailing understanding at present is that humans have one primary sweet taste receptor (i.e. heterodimer of two G-protein coupled receptors, the T1R2-T1R3). (107, 123) Both the T1R2 and T1R3 dimers entail a large extracellular area (i.e. Venus fly trap domain), which is connected to the transmembrane via a cysteine-rich domain. (111) It has been suggested that the Venus flytrap domain of T1R2 targets a large variety of sweet substances (natural sweeteners and most of the NNS); the Venus flytrap domain of T1R3 targets other NNS, such as cyclamate and the sweet receptor blocker, lactisole; and the cysteine-rich domains activate sweet proteins. (95, 111) In the present study there were strong correlations between DT and RT of all caloric sweeteners (sucrose, glucose, fructose, erythritol), and also between DT and RT of the NNS (sucralose, Rebaudioside A). However, the DT and RT of caloric sweeteners and NNS were weakly correlated suggesting at least some independence between the two groups at lower concentrations. This may be due to differences in downstream signalling pathways, or even differences in receptor kinetics as a result of binding to different sites of the sweet taste receptor. (106, 111)
Chapter 3 – Study 1(a) Psychophysical Evaluation of Sweetness Functions across Multiple Sweeteners

The lack of correlation between DTs and RTs of caloric sweeteners and NNS may also be partly explained from the available data comparing NNS and caloric sweeteners in brain studies. (146-148) These data suggest that there is only one primary sweet taste receptor involved in sensing sweetness, but that some individuals may not be able to sense sweetness from NNS as effectively as from caloric sweeteners, due to impairment in their brain’s sweet reward system. In the past decade, studies using functional magnetic resonance imaging (fMRI) data have revealed that the human brain responds differently to caloric sweeteners and NNS, particularly in the area involved in the reward pathway. (146, 147) It has also been found that, compared to non-habitual consumers of NNS, habitual consumers were found to have greater overall activation in the brain reward pathways to both caloric sweeteners and NNS, further suggesting that NNS may impair and adapt the brain’s capability to detect or sense nutrients. (148, 301)

In contrast, there were strong correlations between the sweetness intensity ratings of caloric sweeteners and NNS, supporting commonality of sweet mechanism throughout the perceptual range. This result does support current knowledge of the sweet taste transduction mechanism, in which there is only one primary sweet taste receptor (T1R2-T1R3 heterodimer) responsible for sensing different types of chemical sweeteners at suprathreshold levels.

The finding that, for each sweetener, DTs and RTs were correlated with one another, but not with suprathreshold intensity ratings, suggests added complexity within the sweet taste system. These findings are consistent with previous studies investigating the associations between sucrose taste function, where DTs for sucrose were found to correlate poorly with sucrose suprathreshold intensity ratings. (70, 78) This suggests that there are distinct perceptual stages for sweet threshold and
suprathreshold intensities, with each measure of sweet taste characterising a different component of taste function. (70) There is, therefore, no single measure capable of being a definitive marker of sweet taste function. (54, 70)

In this study, we measured sucrose DT and RT using both the ISO standard method of limits and the more intensive ascending forced choice triangle technique. The present study found significant correlations between sucrose DT and RT using both methods. Thus the results confirm the ISO standard method of limits as a reliable method for the rapid estimation of detection and recognition thresholds for sweet taste (sucrose).

There was large inter-individual variation in sweetness perception. The concentration required to reach DT or RT for a sweetener varied approximately 150 fold across the sample population. There was also large individual difference in perceived sweetness intensity; for example, sucrose (13.7% w/v) was rated 8.8 gLMS by one participant and 40.5 gLMS by another. Inter-individual differences or variability in sweet taste function has been previously observed for sucrose (70, 153-158, 294) and a range of sweeteners. (159-161)

The hypothesis that those who were able to detect and/or recognise low concentrations of sweeteners would also experience higher sweetness intensities was not supported. This relationship was only weakly observed between the DT and suprathreshold intensity measures of erythritol, glucose, and fructose \(i.e. r = 0.26-0.29\), but not for the other sweeteners. These findings are consistent with previous studies investigating the relationships between taste functions in other taste qualities. (54, 69, 70, 76, 179, 302)
3.6 Conclusion

This is the first study to explore the interrelations of DT, RT and suprathreshold perception of sweet taste, in caloric sweeteners and NNS, both within and between individuals. The present data highlight the complexity of human sweetness perception: no single measure of sweet taste function was able to characterise sensitivity, and no one sweet compound was representative of other sweet compounds. The findings are consistent with the proposition of one primary sweet taste receptor for both non-nutritive and caloric sweeteners, with different domains in the receptor.
Chapter Four: Study 1(b) The Associations between Sweet Taste Function, Body Composition, and Dietary Intake in Adults

4.1 Introduction

Increased energy intake, in particular greater intakes of sweet food, is thought to be one of the major contributors to the global rise in being overweight and obese. (31, 303) For example, excessive consumption of sugar-sweetened beverages has been linked to the rising rates of obesity worldwide. (34, 35) The continued increase in the worldwide prevalence of nutrition-related chronic illness such as obesity necessitates an increased understanding of the drivers of food intake. (29, 31, 304, 305)

The sense of taste, one of the traditional five senses (sight, hearing, taste, smell and touch), is activated when nutrients or other chemical compounds stimulate specialised taste receptor cells within the oral cavity. (306) From an evolutionary perspective our taste system functions as a gatekeeper to ingestion ensuring that we consume essential nutrients for survival and functioning, while rejecting potentially harmful or toxic foods. (66) However, research on sweetness, energy intake, and body mass index (BMI) is controversial (see reviews by (307-309)).

The role of taste sensitivity in promoting intake of specific foods or ingredients associated with obesity has long been an area of interest, but with mixed experimental support. (55, 56, 63, 158, 179, 181, 182, 184, 185) In regards to sweet taste, whether or not environmental influences such as habitual diet can alter sweet taste function or
vice versa is still unclear. Some have reported an inverse association between BMI and sweet taste function (decreases in BMI were associated with increased sweet function). (185, 310) For example, a recent study of normal weight (n = 52) and overweight/obese (n = 51) participants found that those with higher BMI had significantly higher DT for sucrose (i.e. higher DT = differentiated the sucrose-containing solution from plain water at a higher concentration level). (188) However, a large body of evidence indicates that there is no significant association between BMI and sweet taste function. (158, 189-195) Similar complexities were also found in studies investigating the link between sweetness liking and BMI, where most data showed no link between hedonics of sweetness and body size. (190, 192, 194, 195, 198, 199, 311) A confounding factor in this area is that non-nutritive sweeteners generally contain only negligible amounts of kilojoules thereby decoupling sweetness from energy value. (49)

Discrepancies between studies may be attributed to differences in the types of sugar and/or psychophysical techniques used to measure sweet taste function (312) as research has shown that no single psychophysical measure reflects taste function in totality. (70) There are three perceptual dimensions of sweet taste function, namely detection threshold, recognition threshold, and suprathreshold intensity, each of which is independent of the other. (54, 70)
4.2 Aims and Hypotheses

4.2.1 Aims

The present study aims to investigate associations between the three common measures of sweet taste function, body composition and dietary intake among adults using multiple sweeteners.

4.2.2 Hypotheses

- No measure of sweet taste function (detection threshold, recognition threshold, or suprathreshold intensity perception) will be associated with body composition and dietary intake for all sweeteners.
4.3 Subjects, Materials, and Methods

4.3.1 Subjects and Study Design

Please refer to Section 3.3.1 and Section 3.3.2 for details regarding subjects and study design.

4.3.2 Participant Training

Prior to using the general Labeled Magnitude Scale (gLMS) to rate taste intensity, participants were trained using the standard protocol outlined by Green et al. (266, 267) except the top of the scale was described as the strongest imaginable sensation of any kind (Appendix G, described in Chapter 2). (75) During the training session, participants were asked to rate the intensity of the perceived sensation relative to a remembered or imagined sensation. Participants were required to rate a list of seven remembered or imagined sensations, such as the warmth of lukewarm water, the pain from biting of the tongue, and the sweetness of fairy floss (known as cotton candy in the USA, or candy floss in the UK) (Appendix H).

4.3.3 Stimuli

As described in Chapter 2, both caloric (glucose monohydrate, fructose, sucrose, erythritol) and non-nutritive sweeteners (NNS) (sucralose, Rebaudioside A) were used to investigate sweet taste (for details of stimuli see Table 4.1).
4.3.4 Detection and Recognition Threshold Determination for Sweet Taste and Suprathreshold Intensity Ratings for Sweeteners

The method for this section has previously been described in Section 3.36 and Section 3.37.

4.3.5 Standardisation of gLMS Usage with Weight Ratings

To standardise gLMS usage within participants, a modified version of Delwiche et al. (313) was adapted for this study, as described in Chapter 2. Significant correlations were found between the overall mean prototypical ratings and overall mean heaviness ratings \( r = 0.28, P < 0.05 \) (see Table 4.1 for concentration of prototypical tastants used for determination of taste intensity perception). As individual ratings for taste intensity and the heaviness of the bottles were assumed to be unrelated, the significant correlation indicated that the gLMS ratings were prone to individual scale-use bias and required standardisation across participants. (54, 70, 313) To determine a personal standardisation factor, the grand mean for heaviness across weight levels and participants was divided by each participant’s average intensity for heaviness. (54) Each individual’s intensity ratings were multiplied by his or her personal standardisation factor for scale-use bias. (54, 313).
Table 4.1 Concentrations (weak, medium, and strong intensity) of tastants used for determination of taste intensity perception for prototypical tastants.

<table>
<thead>
<tr>
<th>Taste quality</th>
<th>Stimulus</th>
<th>Concentration (% w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Weak</td>
</tr>
<tr>
<td>Sweet</td>
<td>Sucrose</td>
<td>3.4</td>
</tr>
<tr>
<td>Salty</td>
<td>Sodium chloride</td>
<td>0.6</td>
</tr>
<tr>
<td>Sour</td>
<td>Citric acid</td>
<td>0.02</td>
</tr>
<tr>
<td>Bitter</td>
<td>Caffeine</td>
<td>0.02</td>
</tr>
<tr>
<td>Umami</td>
<td>Monosodium glutamate</td>
<td>0.05</td>
</tr>
</tbody>
</table>

The concentration series were adapted from Webb et al. (70). Reference chemical details: sucrose (CSR, Yarraville, Australia); sodium chloride (Saxa, Premier Foods Inc, Seven Hills, Australia); citric acid (Ward McKenzie Private Limited, Altona, Australia); caffeine (Sigma Aldrich, Steinham, Germany); and monosodium glutamate (Ajinomoto Cooperation, Tokyo, Japan).

### 4.3.6 Body Composition

As described in Chapter 2, all participants were asked to remove shoes and heavy clothing to ensure accurate measurements. All body composition measurements were measured first thing during the initial and final visits after 1 hour fast (food only). Participants’ body weight was measured to the nearest 0.1 kg using a segmental body composition analyser (TBF-300A) (Tanita Corporation, Tokyo, Japan). Participants’ height was measured to the nearest 0.1 cm using a portable stadiometer (Seca213) (Seca, Eilbek, Germany). All measurements were repeated twice to ensure accuracy. An average of measurements for both height and weight were used to calculate BMI (weight in kg/m²) and determine weight status (i.e. normal weight or overweight/obese). Weight statuses were defined under World Health Organisation BMI classification. (277) Using methods outlined by the Australian Heart Foundation, (278) waist circumference was also measured. Waist circumference was measured twice to the nearest 0.1 cm using an ergonomic circumference measurement tape (Seca201) (Seca, Eilbek, Germany). An average of waist circumference measurements was calculated and used for analysis.
4.3.7 Dietary Intake

The Dietary Questionnaire for Epidemiology Studies Version 2 (DQESV2), (280) a validated version of the Food Frequency Questionnaire (FFQ) (281, 282) developed by Cancer Council Victoria was used to measure each participant’s habitual pattern of food intake (Appendix O). Participants were required to indicate, on average, how many times in the previous year they consumed a number of food and beverage items (74 items) across four categories (i.e. 1) cereal foods; sweets and snacks; 2) dairy products, meats and fish; 3) fruit; and 4) vegetables) with 10 frequency response options ranging from ‘never’ to ‘3 or more times per day’). They were also required to indicate the portion size that they normally consumed. Participants were asked to complete the FFQ within a month from their first visit. Using software based on the Australian nutrient composition database NUTTAB95, (283) analysis was carried out by the Cancer Council Victoria, Australia to assess daily energy and macronutrient intakes.

In addition to the FFQ, participants were asked how often they consumed foods and/or beverages sweetened with NNS (artificial sweeteners, natural NNS) by selecting an appropriate response category from a list of ‘more than once a day’, ‘once per day’, ‘three to six times per week’, ‘once or twice per week’, ‘one to three times per month’, ‘once per month or less’, or ‘never’ (Appendix S). (287) Participants were given examples of commercial products that were sweetened with NNS (both artificial and natural NNS sweetened) such as low-energy carbonated drinks, confectionary and dairy products. Participants were also instructed to include NNS consumed with tea and coffee.
4.3.8 Statistical Analysis

SPSS Version 22.0 software (SPSS, Chicago, IL, USA) was used for the statistical analysis of the data. Data are expressed as means ± standard error of mean (SEM). Descriptive statistics were employed to describe demographic information, sweet taste thresholds and perceived sweetness intensity, dietary intake, and NNS consumption. Sweet taste thresholds and sweet suprathreshold intensity ratings for prototypical tastants and sweeteners were determined as the arithmetic mean of the duplicate measures. For sweet intensity ratings, a geometric mean score of the three ratings (weak, medium, and strong) for all sweeteners was calculated. Over and under reporters for dietary intake were checked for out of range values for energy intake and cases with outlying values (>2 SD from mean energy intake per day) were removed from further dietary analyses. (314) However for BMI and waist circumference, all participants were included in the analysis.

DTs and RTs for each sweetener were treated as grouping variables (quartiles) with participants categorised as more sensitive (1/4), normal sensitive (2/4–3/4), and less sensitive (4/4) to explore differences between continuous (BMI, waist circumference, dietary intake, habitual energy intake, and macronutrient intakes) and categorical (NNS consumption) variables. STs for each sweetener were treated as a grouping variable (quartiles) with participants categorised as those who experienced low intensity (1/4), moderate intensity (2/4–3/4), and high intensity (4/4) to explore differences between continuous (BMI, waist circumference, dietary intake, habitual energy intake, and macronutrient intakes) and categorical (NNS consumption) variables. Sweet taste function for each sweetener was grouped into quartiles to allow comparison of most and least sensitive groupings or those groups who experienced low and high intensity (i.e. 18 sets of quartiles were determined: one for DT for each
sweetener, one for RT for each sweetener, and one for ST for each sweetener). Independent $t$-tests were used to detect differences in habitual energy intake, BMI, and waist circumference between more sensitive and less sensitive participants or those who experienced low and high intensity (lower and higher quartile groups). Chi-square test was used to detect differences in consumption frequency of NNS between sweet taste function groups. Independent $t$-tests were used to detect differences in diet between more sensitive and less sensitive participants or those who experienced low and high intensity, with macronutrient consumption ($i.e.$ percent energy from total sugar, starch, and carbohydrate) as a dependent variable and sweet taste function as the independent variable. Independent $t$-tests were also used to assess differences in terms of weight status between more sensitive and less sensitive participants or those who experienced low and high intensity ($i.e.$ BMI and waist circumference as a dependent variable and sweet taste function as the independent variable). Pearson’s product-moment correlations were conducted to also analyse the relationship between sweet taste function and BMI, waist circumference, and dietary intake. Independent $t$-tests were used to analyse differences in terms of sex between sweet taste function, body composition, and dietary intake. Significance was accepted at $P < 0.01$ to reduce the possibility of making a type I error due to multiple tests being conducted. The $p$-values were not adjusted for multiple comparisons by the application of Bonferroni or other equivalent method, as these approaches can be overly conservative (increasing risk of type II error) and can potentially mask important findings. (315, 316)
4.4 Results

Baseline characteristics of all participants are detailed in Table 4.2.

4.4.1 Sweet Taste Function of Sweet Tastants

Of the 60 participants, $n = 15$ (25%) were asked to complete an additional session due to variability in measurements. There were no significant differences in sweet taste function between male and female participants; therefore, the data are presented together (all $P > 0.01$). The DT and RT means, standard error, and range for all sweeteners are presented in Table 4.3. The geometric mean, standard error, and range of intensity ratings of all sweeteners measured are presented in Table 4.4.

4.4.2 Sweet Taste Function of Body Composition

No significant associations were identified between any measures of sweet taste function (DT, RT, and intensity) with BMI, and waist circumference for all sweeteners tested (all $P > 0.01$). Similarly, when grouped into quartiles, there were no significant differences between more sensitive and less sensitive participants or those who experienced low and high intensity according to their sweet taste function (all sweeteners) for BMI and waist circumference (all $P > 0.01$). When stratified into BMI categories, there were no significant differences in any measure of sweet taste function between the normal weight and overweight/obese participants (all $P > 0.01$).
Table 4.2 *Baseline characteristics of study participants (Mean values and standard errors).*

<table>
<thead>
<tr>
<th></th>
<th>All (n = 60)</th>
<th>Normal Weight (n = 38)</th>
<th>Overweight/Obese (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td></td>
<td>26.5 ± 1.0</td>
<td>26.5 ± 1.4</td>
<td>26.3 ± 1.3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>168.1 ± 1.3</td>
<td>166.1 ± 1.6</td>
<td>171.7 ± 1.8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>68.0 ± 1.8</td>
<td>60.5 ± 1.6</td>
<td>81.0 ± 2.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.9 ± 0.4</td>
<td>22.0 ± 0.3</td>
<td>27.3 ± 0.4</td>
</tr>
<tr>
<td>BMI range (kg/m²)</td>
<td>18.5–32.9</td>
<td>18.5–24.9</td>
<td>25.1–32.9</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>80.5 ± 1.6</td>
<td>73.3 ± 0.9</td>
<td>93.0 ± 2.3</td>
</tr>
<tr>
<td>Waist circumference range (cm)</td>
<td>59.0–112.0</td>
<td>59.0–85.5</td>
<td>73.0–112.0</td>
</tr>
</tbody>
</table>

1 Normal weight, BMI = 18.5–24.9 kg/m²; overweight, BMI = 25–29.9 kg/m²; obese, BMI ≥ 30 kg/m². (277)

Table 4.3 *Taste thresholds (% w/v) of sweet tastants presented as mean, standard error, and range.*

<table>
<thead>
<tr>
<th>Tastant</th>
<th>Detection Threshold (n = 60)</th>
<th>Recognition Threshold (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>Range</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.34 ± 0.04</td>
<td>0.02 – 1.80</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.17 ± 0.03</td>
<td>0.01 – 0.90</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.20 ± 0.03</td>
<td>0.01 – 0.70</td>
</tr>
<tr>
<td>Sucralose (x10⁻³)</td>
<td>0.52 ± 0.07</td>
<td>0.02 – 1.90</td>
</tr>
<tr>
<td>Rebaudioside A (x10⁻³)</td>
<td>0.60 ± 0.06</td>
<td>0.03 – 1.70</td>
</tr>
<tr>
<td>Erythritol</td>
<td>0.28 ± 0.04</td>
<td>0.02 – 0.90</td>
</tr>
</tbody>
</table>
### Table 4.4 Geometric mean (gLMS), standard error, and range sweetness intensity ratings of sweet tastants.

<table>
<thead>
<tr>
<th>Sweetener</th>
<th>Mean ± SEM</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>15.8 ± 1.1</td>
<td>4.9–39.6</td>
</tr>
<tr>
<td>Fructose</td>
<td>15.4 ± 1.0</td>
<td>4.9–31.5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>15.5 ± 1.1</td>
<td>4.1–44.8</td>
</tr>
<tr>
<td>Sucralose</td>
<td>11.6 ± 0.7</td>
<td>2.8–28.5</td>
</tr>
<tr>
<td>Rebaudioside A</td>
<td>11.2 ± 0.8</td>
<td>2.9–25.0</td>
</tr>
<tr>
<td>Erythritol</td>
<td>16.4 ± 1.2</td>
<td>4.7–44.8</td>
</tr>
</tbody>
</table>

#### 4.4.3 Sweet Taste Function and Energy and Macronutrient Intakes

Participants \( n = 4 \) were identified as over-reporters of energy intake (>2 SD ± 10,800.2 kJ), therefore, they were removed from further dietary analyses. When stratified into body sizes, overweight/obese participants had a significantly greater mean total energy intake (13,847.6 (SEM 1,264.8) kJ) in comparison to normal weight participants (9235.3 (SEM 706.4) kJ). There were no other significant differences in dietary intake between normal weight and overweight/obese participants (all \( P > 0.01 \)).

The mean and standard error for energy and macronutrient intakes (in percentages of energy intake) are presented in Table 4.5. No correlations were observed between DT and RT, with mean total energy intake, percent energy from total fat, protein, carbohydrate, sugar, starch, and fibre (all \( P > 0.01 \)). Significant correlations were observed between mean total energy intake and sweetness intensity ratings for Rebaudioside A \( (r = 0.40, P < 0.01) \) and sucralose \( (r = 0.36, P < 0.01) \). However, no significant correlations were identified for mean total energy intake and sweetness intensity ratings for glucose \( (r = 0.30, P = 0.02) \), fructose \( (r = 0.30, P = 0.03) \), sucrose \( (r = 0.26, P = 0.05) \), and erythritol \( (r = 0.23, P = 0.09) \). No correlations were observed between sweetness intensity for all sweeteners and percent energy from total fat, protein, carbohydrate, sugar, starch, and fibre (all \( P > 0.01 \)). When grouped
into quartiles, Tukey post hoc analyses revealed no significant differences between more sensitive and less sensitive participants or those who experienced low and high intensity (lower and higher quartiles) according to their sweet taste measures and the macronutrients investigated (all $P > 0.01$). No robust differences were observed between male and female participants in terms of the associations between sweetness intensity and energy intake (unreported).

Table 4.5 Mean energy intake and macronutrient intakes (in percentages of energy intake) presented as mean and standard error.

<table>
<thead>
<tr>
<th></th>
<th>All ($n = 56$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>Total energy (kJ)</td>
<td>10,800.2 ± 692.9</td>
</tr>
<tr>
<td>Total fat (%)</td>
<td>35.1 ± 1.9</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>20.1 ± 0.8</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>44.9 ± 1.9</td>
</tr>
<tr>
<td>Sugar (%)</td>
<td>13.6 ± 0.8</td>
</tr>
<tr>
<td>Starch (%)</td>
<td>26.1 ± 1.2</td>
</tr>
<tr>
<td>Fibre (%)</td>
<td>4.3 ± 0.2</td>
</tr>
</tbody>
</table>

### 4.4.4 Sweet Taste Function and Consumption of Added Sugar and Specific Sugar-Sweetened Foods

The mean and standard error for consumption of added sugar and specific sugar-sweetened foods (sweet biscuits, cakes, flavoured milk, fruit spreads, ice-cream, chocolate, bread, and breakfast cereals) in grams are presented in Table 4.6. No robust associations between measures of sweet taste function, sugar, and specific sugar-sweetened foods were observed. When grouped into quartiles (DT, RT, sweetness intensity), there were no significant differences between more sensitive and less sensitive participants or those who experienced low and high intensity for all sweeteners in terms of consumption of added sugar and specific sugar-sweetened foods (all $P > 0.01$). There were no significant differences in terms of intake of added
Chapter 4 – Study 1(b) The Associations between Sweet Taste Function, Body Composition, and Dietary Intake in Adults

sugar and specific sugar-sweetened foods between overweight/obese subjects and normal-weight participants (all $P > 0.01$).

Table 4.6 Consumption of added-sugar and specific sugar-sweetened foods in grams. $^{a,b,c}$

<table>
<thead>
<tr>
<th></th>
<th>All $(n = 56)$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grams/day</td>
</tr>
<tr>
<td>Sugar</td>
<td>$9.2 \pm 2.2$</td>
</tr>
<tr>
<td>Sweet biscuits</td>
<td>$8.2 \pm 1.4$</td>
</tr>
<tr>
<td>Cakes</td>
<td>$10.1 \pm 1.6$</td>
</tr>
<tr>
<td>Flavoured milk</td>
<td>$1.7 \pm 0.3$</td>
</tr>
<tr>
<td>Fruit spreads (jam)</td>
<td>$2.8 \pm 0.6$</td>
</tr>
<tr>
<td>Ice-cream</td>
<td>$12.1 \pm 2.5$</td>
</tr>
<tr>
<td>Chocolate</td>
<td>$16.6 \pm 2.2$</td>
</tr>
<tr>
<td>Bread</td>
<td>$95.3 \pm 6.3$</td>
</tr>
<tr>
<td>Breakfast cereals $^c$</td>
<td>$21.0 \pm 3.3$</td>
</tr>
</tbody>
</table>

$^a$ All values are presented as the mean ± SEM. $^b$ Sugar: calculated from teaspoons of sugar used per day. $^c$ Breakfast cereals: includes all bran, branflakes, weet-bix, cornflakes, porridge and muesli.

4.4.5 Sweet Taste Function and Consumption of Non-Nutritive Sweeteners

Most participants did not consume artificial sweeteners ($76.8\%, n = 43$) or natural NNS ($96.4\%, n = 54$) in foods and beverages. Of the participants who did consume artificially sweetened foods and beverages ($n = 43$), nine reported consuming them, on average, three to six times per week, and four reported consuming them at least once per day. There were no significant differences between measures of sweet taste function and frequency of consumption of artificially sweetened foods and beverages (all $P > 0.01$). There were no significant differences in terms of frequency of consumption of artificial and natural NNS between normal weight and overweight/obese participants (all $P > 0.01$).
Chapter 4 – Study 1(b) The Associations between Sweet Taste Function, Body Composition, and Dietary Intake in Adults

4.5 Discussion

To our understanding, this was the first comprehensive study to investigate if multiple measures of sweet taste function using a range of sweeteners were related to body composition measurements or dietary intake. Overall sweet taste function was not associated with body composition measurements or dietary intake, except for mean total energy intake, where moderate correlations were found between sweetness intensity for NNS, Rebaudioside A and sucralose. A trend towards significance was also found between total energy intake and sweetness intensity for all the other sweeteners as well.

Overall, the current findings indicate that sweet DTs and RTs are not associated with dietary intake and body weight. The role of sweet taste in promoting intake of foods or ingredients associated with obesity has long been an area of interest, but with mixed experimental support. (312) The present findings provide no experimental evidence for a relationship between measures of sweet taste function and body size (i.e. (190, 192, 194, 195, 198, 199, 311)) and between most measures of sweet taste function and dietary intake. (158)

The moderate and near significant relationships observed between energy intake and sweetness intensity of two NNS, suggests that intensity ratings are more appropriate when assessing sweet taste associations with energy intake in comparison to sweetness DT and RTs. Similarly, one recent small-randomised controlled trial looked at the effect of reducing intake of simple sugars on DT and ST intensity for sucrose. (116) In the study, 13 participants completed a three-month low-sugar diet and 16 participants (control group) remained on their normal diet. (116) No significant changes in sweet DT were found during the intervention in both groups, but the low-sugar diet group rated sweet puddings as more intense in months two and three of the
A similar, but weaker effect on rated sweetness intensity for flavoured beverages was also found. This supports the general school of thought on psychophysical technique comparisons, where taste thresholds have previously been found to have limited utility in predicting experiences in the real world as threshold measures do not depict the dynamic range of sensory function. Thus, the comparison of the ability of an individual to detect and recognise sweetness from a very small amount of sugar/sweet stimuli may not be as relevant in terms of understanding food behaviour, when most of the sweet and high-energy foods are within the sweetness intensity perception range. As absolute taste threshold measures are time consuming to complete, sweetness perceptions may seem to be a more time efficient method to assess relationships between individual sweet taste function and energy intake.

There are possible explanations for the lack of association between sweetness perception and body composition. First, one could argue that perception of tastant solutions in a laboratory setting bears little relevance to actual intake of real food in everyday life. The impact taste perception has on, especially among adults, is not well understood. For example, sweet liking and aversions are not always direct predictors of intake, and they do not always associate sweetness with liking. Therefore, it has proved difficult to link adult taste liking with body sizes and diet, whether in a laboratory setting or in the real world. In truth, the proportion of sugar in an individual’s diet can be driven by many factors ranging from molecular biology to socio-economic factors such as education level and income. Second, as obesity has been associated with diets containing high levels of fat and sugar, sweet food choices may be influenced to some extent by the participant’s sensitivity and/or preference for fatty foods.
In addition, it is possible to have a diet that is considered to include many high-energy sweet foods, but without the energy coming from sweetness (e.g. energy bars/muesli bars sweetened with NNS, baked goods sweetened with NNS, etc.). As we only measured frequency of consumption of NNS, we do not have data on the quantity and the types of foods that participants consumed that were sweetened with NNS. Furthermore, it is also possible that participants were unaware of the NNS that may be present in foods that they consumed. It is also important to acknowledge that there are large individual differences in preference and desire for sweetness, (172, 173) which could potentially account for the lack of associations between sweet taste and dietary energy relating to sugar consumption. Some people may like increasing concentrations of sucrose, (176, 177) whereas some people may only like increasing concentrations of sucrose up to a bliss point (inverted U-shape) whereby liking decreases as sucrose concentration increases beyond this point. (178, 326-328)

There are some limitations that need to be considered when considering the results. Food Frequency Questionnaires may not accurately reflect diet, and are prone to under and over-reporting. Moreover, there are numerous sweetener options currently available and while the authors used multiple sweeteners in this study there were other sweeteners available that were not used which may have altered the results. The unequal distribution between males and females in the BMI groups may also be a limitation of this study.
4.6 Conclusions

An individual’s ability to detect and recognise a range of sweeteners did not play a role in sweet food consumption, NNS consumption, or more generally the dietary intake of adults. Sweetness intensity from two NNS was associated with energy intake indicating that intensity measures might be more appropriate when assessing associations with total energy intake. There were no associations found between body composition measurements and sweet taste function across a range of sweeteners. Supporting this, there were no differences in sweet taste function between lean and overweight/obese participants. Although all measures of sweet taste function differed between individuals for all sweeteners, oral sweet taste function does not appear to have any robust influence on body composition measurements or dietary intake.
Chapter Five: Study 2(a) Evidence Supporting Oral Sensitivity to Complex Carbohydrates Independent of Sweet Taste Sensitivity in Humans\(^1\)

\(^1\)This study is currently under review at Plos One Journal as ‘Evidence Supporting Oral Sensitivity to Complex Carbohydrates Independent of Sweet Taste Sensitivity in Humans’.

5.1 Introduction

Complex carbohydrates and simple sugars are two essential sources of energy in our diet. Except for some fruits, complex carbohydrates are more abundant than simple sugars in plants, but it is sugars with their hedonically pleasing sweet taste that are the utmost sought-after carbohydrate. (2) In line with this, there is also growing evidence demonstrating that rodents (e.g. rats, mice, gerbils, hamsters) and even some non-human primates are attracted to the taste of complex carbohydrates derived from maltodextrin (also known as glucose polymer). (8, 329) These evidence has been summarised in Lapis \textit{et al.} (18, 21) indicating independent taste peripheral physiology for complex carbohydrates and simple sugars, (8, 225) but the taste receptor remains unknown. (235) Furthermore, recent behavioural studies from exercise science support the notion that humans can detect complex carbohydrates within the oral cavity (see Lapis \textit{et al.} (18, 21) and systematic review by e Silva \textit{et al.} (271)).

Two recent human psychophysical studies propose that humans may perceive complex carbohydrates independent of sweet taste (\textit{i.e.} glucose and sucrose were significantly correlated with each other, but not with complex carbohydrates). (18, 19)
For example, Lapis et al. (20) found that humans \( n = 25 \) were able to discriminate complex carbohydrate solutions (glucose oligomers but not glucose polymers) from water even when the sweet taste receptors (T1R2-T1R3 heterodimer) are inhibited by lactisole treatment. Lactisole is a sweet taste blocker known to bind to a pocket in the transmembrane region of the T1R3 and thus inhibits the sweet taste perception of sugars, proteins, and non-nutritive sweeteners. (109) While the human taste perception of complex carbohydrate (starch) hydrolysis has been well investigated by Lapis et al. (18, 20), it needs replication and also extension.

At present, there is also only one known human psychophysical study that has investigated if oral sensitivity to complex carbohydrates is independent of some of the other basic tastes (i.e. sweet and salty taste). Lapis et al. (18) showed no significant correlations between the intensity ratings of glucose (sweet taste), sucrose (sweet taste), and sodium chloride (salty taste) with the intensity ratings of complex carbohydrates. However, it is still uncertain if this measure is independent of the intensity ratings of the remaining common prototypical tastes stimuli such as monosodium glutamate (umami taste), caffeine (bitter taste), and citric acid (sour taste). As each measure of taste function (detection threshold, recognition threshold, and suprathreshold intensity perception) represents a different dimension of the sense of taste, there is currently no single method to measure taste function in totality. (54, 70) Even though the perceptual relationship between a range of caloric and non-nutritive sweeteners have been reported, there is currently no single study that has investigated the relationships between complex carbohydrates and multiple sweeteners (caloric and non-nutritive) using a range of psychophysical measures within a single group of individuals.
5.2 Aims, Hypotheses, and Terminologies

5.2.1 Aims

The aim of this study is to investigate if humans can perceive two complex carbohydrates: a soluble starch, maltodextrin, and a soluble fibre, oligofructose.

5.2.2 Hypotheses

- Participants will have detection threshold and will be able to perceive intensity for complex carbohydrate (maltodextrin);
- There will be significant individual variance in oral complex carbohydrate sensitivity. Participants will be able to be classified into more sensitive/experienced higher intensity, normal sensitive/experienced moderate intensity, and less sensitive/experienced lower intensity groups according to the sensitivities of complex carbohydrate (maltodextrin).
5.2.3 Terminologies

Although the terminology “polysaccharide taste” has been recommended by Sclafani (2) to denote starch-derived saccharides containing three or more glucose units, it can be confusing as the word “polysaccharide” is generally used to describe complex carbohydrates comprising more than ten monosaccharide units organised in chains. The word “oligosaccharide taste” (two to nine monosaccharide units) would be the more appropriate terminology, nonetheless, it is not user friendly and it is unknown if perception of oligosaccharides is independent of textural differences. Therefore, at this stage of knowledge we recommend the use of “oral sensitivity to complex carbohydrate”, which correctly comprises all types of complex carbohydrates and derivatives including fibres (e.g. oligofructose), while not diminishing the prospect that oral perception of complex carbohydrates could be due to textural differences. Whilst dietary “carbohydrate” is an umbrella term for the monosaccharide and disaccharide sugars as well as starches and fibres, the term “sweet taste” has been collectively used to indicate sweetness. Thus “oral sensitivity to complex carbohydrate” would at the current state of knowledge be as correct as possible without oversimplifying tasting complex carbohydrates, but not easily confused with other sensations such as sweetness.

5.3 Subjects, Materials, and Methods

5.3.1 Study Design

This study comprised a total of 28 laboratory-based sessions in which data on two measures of taste perception routinely used in chemosensory research was collected: (1) detection threshold (DT) and (2) suprathreshold intensity rating (ST).
These measures were determined for all participants for each of two complex carbohydrates, six sweeteners, and prototypical stimuli for sour, salty, umami, and bitter during a total of 28 sessions. Oral complex carbohydrate sensitivity (DTs and STs; maltodextrin, oligofructose) measurements were collected over seven separate testing sessions (> one hour apart) on each of four non-consecutive days (two days for each complex carbohydrate). All sweet taste measurements were collected in duplicates (two sessions per day over six non-consecutive days). Measurements for the prototypical stimuli were collected in duplicates (one session), and participants also participated in a general Labeled Magnitude Scale (gLMS) training session. If there were more than three concentration steps between the repeated measures, participants attended another session to complete the assessment. Participants in the present chapter were part of a larger study focusing on the psychophysics of sweet taste measures for the six sweeteners. (270) DT and ST tasks were conducted in computerised, partitioned sensory booths in the Centre for Advanced Sensory Science using Compusense Cloud Software as part of the Compusense Academic Consortium (Compusense Inc., Ontario, Canada). Filtered deionised water was used as an oral rinsing agent. Participants were instructed to rinse their mouths with filtered deionised water for five seconds before beginning each task and between each sample set. To eliminate any visual and olfactory input, all testing sessions were conducted under red lighting, and participants were asked to wear nose clips during testing. All solutions were served at room temperature, with a three-digit code allocated to each sample.
5.3.2 Subjects

Participants [(n = 34): 16 males, age 26.2 ± 0.4 years (range, 24 - 30 years), BMI 25.2 ± 0.9 kg/m² (range, 18.9 – 30.0 kg/m²); 18 females, age 29.4 ± 2.1 years (range, 24-55 years), BMI 24.3 ± 0.8 kg/m² (range, 20.0 – 29.6 kg/m²)] were recruited via email and flyer distribution from locations adjacent to the Melbourne Burwood campus of Deakin University, Australia. These participants also participated in Study 1. Potential participants were excluded if they were: (1) smokers; (2) pregnant or lactating; (3) taking any prescription medication that may interfere with their ability to taste; or (4) had a history of food allergies that may interfere with the study. Participants were asked to refrain from eating, drinking (except room temperature water), brushing their teeth, and chewing gum for one hour prior to testing. All participants gave written informed consent and were compensated for their participation. This study was approved by the institutional review board regulations of Deakin University (HEAG_H_182_2014). The experimental protocol was also registered under the Australian New Zealand Clinical Trials Registry (ACTRN12613000701729), www.anzctr.org.au. This study also complies with the Declaration of Helsinki for Medical Research involving Human Subjects.

5.3.3 Participant training

As described in Chapter 2, prior to using the general Labeled Magnitude Scale (gLMS) to rate taste intensity, participants were trained using the standard protocol outlined by Green et al. (266, 267) except the top of the scale was described as the strongest imaginable sensation of any kind. (75) The 100-point scale comprised the following adjectives: ‘no sensation’ = 0, ‘barely detectable’ = 1.5, ‘weak’ = 6,
‘moderate’ = 17, ‘strong’ = 35, ‘very strong’ = 52, and ‘strongest imaginable’ = 100. (75) Scales with only adjectives (not numbers) were presented to participants. During the training session, participants were taught to rate the intensity of the perceived sensation relative to a remembered or imagined sensation when using the gLMS scale.

5.3.4 Stimuli

Maltodextrin and oligofructose were used to investigate oral complex carbohydrate sensitivity (DTs and STs for both complex carbohydrates; for details of stimuli see Table 5.1; Chapter 2). Maltodextrin with a dextrose equivalent (DE) of five was used in this study as it contains the lowest possible amount of free sugar (glucose, maltose) yet is soluble in water. DE is a measure of the percentage of reducing sugars relative to glucose on a dry basis. (269)

Both caloric (glucose, fructose, sucrose, and erythritol) and non-nutritive sweeteners (NNS) (sucralose and Rebaudioside A) were used to investigate sweet taste (for details of stimuli see Table 5.1). Prototypical stimuli (sodium chloride, citric acid, caffeine, and monosodium glutamate (MSG)) were used to investigate taste function for salty, sour, bitter, and umami (for details of stimuli see Table 5.2). All samples were prepared fresh on the day of testing using filtered deionised water (Cuno Filter Systems FS117S, Meriden, CT, USA) and stored in glass beakers at room temperature (20 ± 1 °C).
Table 5.1 *Complex carbohydrate and sweetener concentrations used for determination of detection thresholds.*

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Concentration (% w/v)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltodextrin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Amount of Glucose in Maltodextrin (x10^-3)</em></td>
<td>0.3 0.5 0.9 1.6 2.8 5.8 9.0 15.9 28.4 50.5 90.0 160.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Amount of Total Sugars in Maltodextrin (x10^-3)</em></td>
<td>1.1 1.6 3.0 5.6 9.8 17.6 31.4 55.7 99.4 176.7 314.7 560.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oligofructose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Amount of Fructose in Oligofructose (x10^-3)</em></td>
<td>0.5 0.8 1.5 2.8 4.9 8.8 15.6 27.8 49.7 88.3 157.3 280.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Amount of Total Sugars in Oligofructose (x10^-3)</em></td>
<td>1.2 1.8 3.3 6.6 10.5 18.9 33.6 59.7 106.5 189.3 337.2 600.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.02 0.03 0.05 0.09 0.1 0.2 0.4 0.6 1.1 1.8 2.9 4.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.01 0.02 0.03 0.05 0.08 0.1 0.2 0.3 0.5 0.9 1.5 2.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.01 0.02 0.03 0.06 0.09 0.1 0.2 0.2 0.4 0.7 1.2 1.8 3.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucralose (x 10^-3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.02 0.04 0.06 0.09 0.1 0.2 0.2 0.4 0.7 1.1 1.9 3.1 5.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rebaudioside A (x 10^-3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.03 0.05 0.09 0.1 0.2 0.3 0.6 1.0 1.7 2.8 4.6 7.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythritol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.02 0.03 0.05 0.08 0.1 0.2 0.3 0.6 0.9 1.6 2.6 4.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The concentration series for sucrose was adapted from ISO3972 (265) The concentration series for maltodextrin, oligofructose, glucose, fructose, sucralose, erythritol, and Rebaudioside A were prepared with successive 0.25 log dilution steps. Reference chemical details: maltodextrin (Star-Dri 5, Tate & Lyle Ingredients Americas, USA); oligofructose (Fibrulose F97, CoSucra-Groupe Warcoing, Belgium); glucose (The Melbourne Food Depot, Melbourne, Australia); fructose (The Melbourne Food Depot, Melbourne, Australia); sucrose (CSR, Yarraville, Australia); sucralose (The Melbourne Food Depot, Melbourne, Australia); Rebaudioside A (AuSweet, Melbourne, Australia); and erythritol (AuSweet, Melbourne, Australia). Calculation of the amount of common and total sugars in maltodextrin and oligofructose concentrations were according to the report of analysis by the Australian Government National Measurement Institute from samples used in this study, where there were a total of 2.8g/100g (2.8% w/w) of free sugars for the maltodextrin (Glucose: 0.8% w/w) and 3.0g/100g (3.0% w/w) of free sugars for the oligofructose (Fructose: 1.4% w/w).
Table 5.2 *Stimulus concentrations used for prototypical threshold testing (% w/v).*

<table>
<thead>
<tr>
<th>Taste quality</th>
<th>Stimulus</th>
<th>Concentrations (% w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Salty</td>
<td>Sodium chloride</td>
<td>0.01</td>
</tr>
<tr>
<td>Bitter</td>
<td>Caffeine</td>
<td>0.006</td>
</tr>
<tr>
<td>Sour</td>
<td>Citric acid</td>
<td>0.013</td>
</tr>
<tr>
<td>Umami</td>
<td>Monosodium glutamate (MSG)</td>
<td>0.008</td>
</tr>
</tbody>
</table>
5.3.5 Analysis of Common Sugars in Maltodextrin and Oligofructose Samples

To determine if the maltodextrin and oligofructose used in this study would be suitable products, four percent \( w/v \) maltodextrin and oligofructose solutions were prepared for High Performance Liquid Chromatography (HPLC), as described in Chapter 2. The complex carbohydrate extracts were clarified with 25mL acetonitrile and filtered through a 0.45um filter into a 2mL vial. To determine the amount of common sugars in samples, filtered solutions were analysed by HPLC using amino column with an acetonitrile/water mobile phase containing salt and refractive index detection. Quantitation was made using a standard solution containing known amount of fructose, glucose, sucrose, maltose and lactose. Samples were measured in duplicate.

There were a total of 2.8g/100g (2.8% \( w/w \)) of free sugars for the maltodextrin (Glucose: 0.8% \( w/w \)) and 3.0g/100g (3.0% \( w/w \)) of free sugars for the oligofructose (Fructose: 1.4% \( w/w \)) used in this study (Table 5.3). Detailed in Table 5.1 are the amounts of common sugars and total sugars (% \( w/v \)) present in each of the complex carbohydrate DT concentrations.

5.3.6 Detection Threshold Determination for Sweet Taste and Oral Sensitivity to Complex Carbohydrates

Detailed in Table 5.1 are the concentration ranges used to assess DTs for sweet taste and oral complex carbohydrate sensitivity, as discussed in Chapter 2. The concentration series for sucrose was adapted from ISO3972 (265); concentrations for
the remaining sweeteners and complex carbohydrates were prepared with successive 0.25 log dilution steps. (270)

Initial starting concentrations for sweeteners were determined through informal bench-top testing, based on modified findings of matching sweetness intensity ratios published by Keast et al. (262). Concentrations for complex carbohydrates were derived based on previous published findings of perceptually distinctive oral sensation concentrations (i.e. see Lapis et al. (18) and systematic literature review by e Silva (271)) and without perceivable viscosity. After pilot testing, a concentration range between 0.04-20.0 percent (w/v) was used to measure DT levels for complex carbohydrates. As maltodextrin is similar in oral sensation and appearance to oligofructose, similar concentrations were used for both complex carbohydrates. (272-275) DTs for each of the sweeteners and complex carbohydrates were determined using ascending forced choice triangle methodology, (263, 264) in which the participants were provided with sets of three 25 mL samples, two of which were controls (filtered deionised water) and one contained sweetener/complex carbohydrate, in ascending order from the lowest to the highest concentration level. DT was defined as the concentration of sweetener/complex carbohydrate required for a participant to correctly identify the sweetened/complex carbohydrate sample, as the odd one out in three consecutive sample sets at one concentration level. (263)
5.3.7 Detection Threshold Determination for Salty, Sour, Bitter, and Umami Tastes

DT was determined using the procedure outlined in the International Standards Organisation (ISO) Method of Investigating Sensitivity of Taste, as described in Chapter 2. (265) Table 5.2 gives the nine concentrations used for each taste quality (the ninth concentration being presented only when participants were unable to differentiate the solutions from water in the previous eight concentrations). (265) The eight samples from each taste quality were served in ascending concentration (15 mL per sample, in accordance with the standard ISO method), and each taste quality was presented to participants independently. Participants were unaware of the presentation order but were informed of the possible taste qualities. Participants were instructed to taste each sample for five seconds then expectorate and record whether: there was an absence of taste (water-like); a taste was identified but not recognised; or a taste quality was perceived. (265) DT was defined as the concentration at which the participants selected the ‘taste identified, but unknown taste quality’ response. (265)
5.3.8 Suprathreshold Intensity Ratings for the Sweeteners, Complex Carbohydrates, and Prototypical Tastants

As described in Chapter 2, three concentrations (weak, medium, and strong) and a control (blank) solution were prepared to determine perceived ST for each prototypical tastant and sweetener (Table 5.4). For complex carbohydrates, four concentrations of complex carbohydrate solutions (weak, medium, medium-strong, strong) and a control (blank) solution were prepared (Table 5.5). These concentrations were derived through informal bench-top testing (ascending taste intensity), but were similar to the concentrations outlined by Webb et al. (70). The concentrations for each stimulus ranged from ‘weak’ to ‘strong’ on the gLMS. Each stimulus was presented to participants independently (sets), but in a randomised order.

5.3.9 Standardisation of gLMS Usage with Weight Ratings

To standardise gLMS usage within participants, a modified version of the method used by Delwiche et al. (268) was adapted for this study, as discussed in Chapter 2.
Table 5.4 Concentrations (weak, medium, and strong intensity) of prototypical tastants and sweeteners used for determination of suprathreshold taste intensity.

<table>
<thead>
<tr>
<th>Taste quality</th>
<th>Stimulus</th>
<th>Concentration (% w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Weak</td>
</tr>
<tr>
<td>Salty</td>
<td>Sodium chloride</td>
<td>0.6</td>
</tr>
<tr>
<td>Bitter</td>
<td>Caffeine</td>
<td>0.02</td>
</tr>
<tr>
<td>Sour</td>
<td>Citric acid</td>
<td>0.02</td>
</tr>
<tr>
<td>Umami</td>
<td>Monosodium glutamate (MSG)</td>
<td>0.05</td>
</tr>
<tr>
<td>Sweet</td>
<td>Glucose</td>
<td>5.3</td>
</tr>
<tr>
<td>Sweet</td>
<td>Fructose</td>
<td>2.9</td>
</tr>
<tr>
<td>Sweet</td>
<td>Sucrose</td>
<td>3.4</td>
</tr>
<tr>
<td>Sweet</td>
<td>Sucralose (x10^-3)</td>
<td>5.7</td>
</tr>
<tr>
<td>Sweet</td>
<td>Rebaudioside A (x10^-3)</td>
<td>8.6</td>
</tr>
<tr>
<td>Sweet</td>
<td>Erythritol</td>
<td>5.7</td>
</tr>
</tbody>
</table>
Chapter 5 – Study 2(a) Evidence Supporting Oral Sensitivity to Complex Carbohydrates Independent of Sweet Taste Sensitivity in Humans

Table 5.5 Concentrations (weak, medium, and strong intensity) of complex carbohydrates used for determination of suprathreshold intensity.

<table>
<thead>
<tr>
<th></th>
<th>Concentration (% w/v)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weak</td>
<td>Medium</td>
<td>Medium-Strong</td>
<td>Strong</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>3.6</td>
<td>6.3</td>
<td>11.2</td>
<td>20.0</td>
</tr>
<tr>
<td>Oligofructose</td>
<td>3.6</td>
<td>6.3</td>
<td>11.2</td>
<td>20.0</td>
</tr>
</tbody>
</table>

To control for idiosyncratic scale usage, participants were asked to rate the heaviness of six, visually identical weights (opaque bottles filled with sand and stone and completely wrapped in aluminium foil; weights of 53, 251, 499, 724, 897, and 1127g) (Appendix I). Participants were asked to hold out their non-dominant hand palm up, while the experimenter placed the weighted bottle on the palm of the hand. Participants were instructed to rate the heaviness of each weight using the gLMS.

There was a significant correlation between the overall mean prototypical ratings and overall mean heaviness ratings ($r = 0.39, P < 0.05$). Assuming that the intensity ratings of prototypical tastants and the heaviness of the bottles were unrelated, the significant correlation indicates that the gLMS ratings were subject to differences in individual scale-use and thus require standardisation across participants. (54, 70, 268) To determine a personal standardisation factor, the grand mean for heaviness across weight levels and participants was divided by each participant’s average intensity for heaviness. (54) Each individual’s prototypical taste intensity and sweetness intensity ratings were multiplied by his or her personal standardisation factor for scale-use bias. (54, 268)
5.3.10 Statistical Analysis

Statistical analysis was performed using IBM SPSS statistical software version 23.0 (SPSS, Chicago, IL, USA). Data are presented as means with standard errors of mean (SEM). The DTs and STs were determined as the arithmetic mean of the repeated measures, and Intraclass Correlation Coefficient (ICC) was used as an indicator of reliability. For STs, the geometric mean score of the three/four ratings (weak, medium, medium-strong, and strong) was calculated. (70) Spearman’s rank correlation coefficient was calculated between distinct measures of taste function to minimise type I error. In order to simplify the data presentation for correlations between DTs and STs, negative $r$-values were converted to positive and vice versa. (54, 270) The criterion for statistical significance was set at $P < 0.05$.

DTs for each complex carbohydrate, sweetener, and prototypical tastant were treated as grouping variables (tertiles) with participants categorised as more sensitive (1/3), normal sensitive (2/3), and less sensitive (3/3) to explore relationships between oral complex carbohydrate sensitivity, sweet taste function, and prototypical taste function. STs for each complex carbohydrate, sweetener, and prototypical tastant were treated as grouping variables (tertiles) with participants categorised as those who experienced low intensity (1/3), moderate intensity (2/3), and high intensity (3/3) to explore relationships between oral complex carbohydrate sensitivity, sweet taste function, and prototypical taste function. DTs and STs for each complex carbohydrate, sweetener, and prototypical tastant were grouped into tertiles to allow comparison of most and least sensitive groupings or those groups who experienced low and high intensity (i.e. 24 sets of tertiles were determined: one for DT for each complex carbohydrate, sweetener, and prototypical tastant, and one for ST for each complex carbohydrate, sweetener, and prototypical tastant). (270)
5.4 Results

5.4.1 Test-retest Reliability of Complex Carbohydrates

All measured thresholds and suprathreshold intensities proved reliable. For maltodextrin, the test-retest correlation reached significance for both detection, \( r = 0.91-0.95 \) (ICC = 0.95), \( P < 0.001 \), and suprathreshold intensity perception, \( r = 0.50-0.98 \) (ICC = 0.66-0.85), \( P < 0.001 \). Similarly, for oligofructose, the test-retest correlation reached significance for both detection, \( r = 0.88-0.97 \) (ICC = 0.95), \( P < 0.001 \), and suprathreshold intensity perception, \( r = 0.47-0.96 \) (ICC = 0.51-0.94), \( P < 0.001 \).

5.4.2 Oral Detection Thresholds of Complex Carbohydrates and Relationship with Taste Detection Thresholds of Sweeteners

There were no significant differences in both oral complex carbohydrate sensitivity and sweet taste function between male and female participants; therefore, the data are presented together (all \( P > 0.05 \)). Mean (\( \pm \) SEM) DT values for the complex carbohydrates and sweeteners are presented in Table 5.6. There was large individual variation among the participants, for example DT for maltodextrin ranged from 0.04 to 6.31 % w/v (Figure 5.1a).

The DTs of complex carbohydrates (maltodextrin, oligofructose) were strongly correlated with one another (\( r = 0.94, P < 0.001 \); Table 5.6; Figure 5.2). Similarly, caloric sweeteners (glucose, fructose, sucrose, and erythritol) were strongly correlated with one another (\( r = 0.84-0.93, P < 0.001 \)), as were NNS (sucralose, Rebaudioside...
A) \( r = 0.68, P < 0.001 \). (270) To verify that free sugars in complex carbohydrate solutions were below DT, if a participant is able to detect glucose in water (DT) at the lowest concentration (0.02% \( w/v \)), potentially that would trigger detection for maltodextrin solution at step 6 (total sugars in maltodextrin: 0.018% \( w/v \)). However, there were no significant correlations between the DTs of the complex carbohydrates (maltodextrin, oligofructose) and the sweeteners (glucose, fructose, sucralose, Rebaudioside A, erythritol) (all \( P > 0.05 \)). (270) This suggests that threshold sensitivity to complex carbohydrates (maltodextrin, oligofructose) does not predicate that the person will be sensitive to the sweetness of sweeteners.

Table 5.6 Detection thresholds for complex carbohydrates and sweeteners (% \( w/v \), including mean, standard error of mean (SEM), and range.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SEM</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltodextrin</td>
<td>1.7 ± 0.3</td>
<td>0.04-6.3</td>
</tr>
<tr>
<td>Oligofructose</td>
<td>1.8 ± 0.4</td>
<td>0.04-7.7</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.3 ± 0.05</td>
<td>0.02-1.1</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.2 ± 0.03</td>
<td>0.01-0.6</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.2 ± 0.05</td>
<td>0.01-1.0</td>
</tr>
<tr>
<td>Sucralose ( \times 10^{-3} )</td>
<td>0.4 ± 0.07</td>
<td>0.02-1.2</td>
</tr>
<tr>
<td>Rebaudioside A ( \times 10^{-3} )</td>
<td>0.7 ± 0.09</td>
<td>0.05-1.7</td>
</tr>
<tr>
<td>Erythritol</td>
<td>0.3 ± 0.05</td>
<td>0.02-1.0</td>
</tr>
</tbody>
</table>
Figure 5.1 Frequency distributions of detection thresholds for: (a) maltodextrin, (b) oligofructose, (c) glucose, (d) fructose, (e) sucrose, (f) sucralose, (g) Rebaudioside A, (h) erythritol.
Continued Figure 5.1 Frequency distributions of detection thresholds for: (a) maltodextrin, (b) oligofructose, (c) glucose, (d) fructose, (e) sucrose, (f) sucralose, (g) Rebaudioside A, (h) erythritol.
Figure 5.2 (1) Spearman rank correlations between detection thresholds of maltodextrin and oligofructose. (2a-d) Correlations between detection thresholds of maltodextrin and caloric sweeteners: (2a) glucose; (2b) fructose; (2c) sucrose; (2d) erythritol. (2e-h) Correlations between detection thresholds of oligofructose and caloric sweeteners: (2e) glucose; (2f) fructose; (2g) sucrose; (2h) erythritol. (3a, 3b) Correlations between detection thresholds of maltodextrin and non-nutritive sweeteners: (3a) sucralose; (3b) Rebaudioside A. (3c, 3d) Correlations between detection thresholds of oligofructose and non-nutritive sweeteners: (3c) sucralose; (3d) Rebaudioside A. The solid line in each graph represents the regression line. *$P<0.05$; **$P<0.001$. 
5.4.3 Suprathreshold Intensities for the Complex Carbohydrates and Relationship with Suprathreshold Intensities for Sweeteners

Figure 5.3 shows the psychophysical functions for all complex carbohydrates and sweeteners. As expected there were monotonic increases in perceived intensity as the concentration of the stimuli was increased. Spearman’s rank correlation revealed a significant relationship between the STs at the four concentrations on a complex carbohydrates’ psychophysical function: (maltodextrin $r = 0.77-0.92$, $P < 0.001$); (oligofructose $r = 0.76-0.95$, $P < 0.001$). Similar relationships were also observed between the STs at the three concentrations on a sweeteners’ psychophysical function: (glucose $r = 0.44-0.86$, $P < 0.001$); (fructose $r = 0.30-0.89$, $P < 0.001$); (sucrose $r = 0.53-0.87$, $P < 0.001$); (erythritol; $r = 0.69-0.93$, $P < 0.001$); (Rebaudioside A; $r = 0.55-0.85$, $P < 0.001$); (sucralose; $r = 0.44-0.87$, $P < 0.001$). Analysis of variance showed significant differences between all incremental steps on the psychophysical functions ($P < 0.05$). This indicates that when a participant is given increasing concentration of a complex carbohydrate or a sweetener (above the DT), there is an ordinal increase in intensity relative to STs across all participants. For each participant, there were strong correlations between the mean STs of complex carbohydrates (maltodextrin, oligofructose) ($r = 0.95$, $P < 0.001$; Figure 5.4). There were also moderate correlations between the STs of complex carbohydrates and sweeteners ($r = 0.48-0.61$, $P < 0.05$).
Chapter 5 – Study 2(a) Evidence Supporting Oral Sensitivity to Complex Carbohydrates Independent of Sweet Taste Sensitivity in Humans

Figure 5.3 Psychophysical curves of the group mean and examples of a participant who experienced high intensity and low intensity for (a) Maltodextrin (b) Oligofructose (c) Glucose (d) Fructose (e) Sucrose (f) Sucralose (g) Rebaudioside A (h) Erythritol. Included in each graph is the mean psychophysical curve as well as an example of a participant who experienced high intensity (highest curve) and a participant who experienced low intensity (lowest curve) for that complex carbohydrate/sweetener. The y-axis is a numerical measure of intensity perception from the gLMS. The x-axis is the actual concentration in % w/v.
Continued Figure 5.3 Psychophysical curves of the group mean and examples of a participant who experienced high intensity and low intensity for (a) Maltodextrin (b) Oligofructose (c) Glucose (d) Fructose (e) Sucrose (f) Sucralose (g) Rebaudioside A (h) Erythritol. Included in each graph is the mean psychophysical curve as well as an example of a participant who experienced high intensity (highest curve) and a participant who experienced low intensity (lowest curve) for that complex carbohydrate/sweetener. The y-axis is a numerical measure of intensity ratings from the gLMS. The x-axis is the actual concentration in % w/v.
Chapter 5 – Study 2(a) Evidence Supporting Oral Sensitivity to Complex Carbohydrates Independent of Sweet Taste Sensitivity in Humans

**Figure 5.4**  (1) Spearman rank correlations of intensity ratings between maltodextrin and oligofructose. (2a-d) Correlations between intensity ratings of maltodextrin and caloric sweeteners: (2a) glucose; (2b) fructose; (2c) sucrose; (2d) erythritol. (2e-h) Correlations between intensity ratings of oligofructose and caloric sweeteners: (2e) glucose; (2f) fructose; (2g) sucrose; (2h) erythritol. (3a, 3b) Correlations between intensity ratings of maltodextrin and non-nutritive sweeteners: (3a) sucralose; (3b) Rebaudioside A. (3c, 3d) Correlations between intensity ratings of oligofructose and non-nutritive sweeteners: (3c) sucralose; (3d) Rebaudioside A. The solid line in each graph represents the regression line. **P<0.001.
5.4.4 Relationships between Oral Detection Thresholds and Suprathreshold Intensities of Complex Carbohydrate Solutions

Significant correlations were observed between DTs and STs for maltodextrin and oligofructose ($r = 0.39-0.53$, $P < 0.05$).

5.4.5 Taste Function of Prototypical Tastants and Relationship with Oral Detection Thresholds and Suprathreshold Intensities of Complex Carbohydrate Solutions

DTs and STs of the four-prototypical tastes are presented in Table 5.7 and Table 5.8. No significant correlations were observed between prototypical taste function (DTs and STs) and DTs and STs of both maltodextrin and oligofructose, except between DT of oligofructose and citric acid (sour) ($r = 0.11$, $P < 0.05$).

Table 5.7 Detection thresholds for four prototypical tastants (% w/v), including mean, standard error of mean (SEM), and range.

<table>
<thead>
<tr>
<th>Detection Threshold</th>
<th>Mean ± SEM</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>0.02 ± 0.002</td>
<td>0.02-0.07</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.015 ± 0.0004</td>
<td>0.013-0.025</td>
</tr>
<tr>
<td>Caffeine</td>
<td>0.007 ± 0.0002</td>
<td>0.006-0.009</td>
</tr>
<tr>
<td>Monosodium glutamate (MSG)</td>
<td>0.012 ± 0.0007</td>
<td>0.008-0.02</td>
</tr>
</tbody>
</table>
5.4.6 Relationships between Oral Complex Carbohydrate Sensitivity, Sweet Taste Function, and Prototypical Taste Function

Participants were stratified into tertile groups according to the complex carbohydrates and sweeteners tested and all taste measures. We observed that those who were able to detect maltodextrin in water at low concentrations (lower tertile; \( n = 8 \)) were also more sensitive to oligofructose. Similarly, those who were able to detect maltodextrin in water at higher concentrations (higher tertile; \( n = 11 \)) were also less sensitive to oligofructose. Interestingly, we also observed that five participants were more sensitive only towards complex carbohydrates (maltodextrin, oligofructose) but were less sensitive to caloric sweeteners (glucose, fructose, sucrose, erythritol). Seven participants were more sensitive towards maltodextrin but not to glucose. Similarly, six participants were more sensitive towards oligofructose but not to fructose. Looking at the concentrations, it is likely that they detected the complex carbohydrates in the sample rather than any free sugars in the complex carbohydrate sample. For example, one participant was able to detect maltodextrin at 0.04% \( w/v \) (Glucose: 0.0003% \( w/v \), total sugars in maltodextrin: 0.0011% \( w/v \)) but only able to detect glucose at 1.1% \( w/v \).

Table 5.8 Suprathreshold intensity ratings for four prototypical tastants on gLMS, given by mean and standard error of mean (SEM).

<table>
<thead>
<tr>
<th>Concentration (% w/v)</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td></td>
</tr>
<tr>
<td>0.6</td>
<td>16.7 ± 2.6</td>
</tr>
<tr>
<td>1.2</td>
<td>24.4 ± 3.6</td>
</tr>
<tr>
<td>2.3</td>
<td>32.8 ± 4.1</td>
</tr>
<tr>
<td>Citric acid</td>
<td></td>
</tr>
<tr>
<td>0.02</td>
<td>21.5 ± 4.7</td>
</tr>
<tr>
<td>0.06</td>
<td>27.3 ± 4.7</td>
</tr>
<tr>
<td>0.13</td>
<td>34.4 ± 4.9</td>
</tr>
<tr>
<td>Caffeine</td>
<td></td>
</tr>
<tr>
<td>0.02</td>
<td>11.6 ± 3.0</td>
</tr>
<tr>
<td>0.04</td>
<td>19.8 ± 3.4</td>
</tr>
<tr>
<td>0.08</td>
<td>30.3 ± 4.1</td>
</tr>
<tr>
<td>Monosodium glutamate (MSG)</td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>11.6 ± 1.5</td>
</tr>
<tr>
<td>0.10</td>
<td>18.0 ± 2.6</td>
</tr>
<tr>
<td>0.20</td>
<td>22.5 ± 3.6</td>
</tr>
</tbody>
</table>
Likewise, one participant was able to detect oligofructose at 0.04% w/v (Fructose: 0.0005% w/v, total sugars in oligofructose: 0.0012% w/v) but only able to detect fructose at 0.57% w/v. For STs, we observed that some participants experienced low intensity (lower tertile; \( n = 2 \)) or high intensity (higher tertile; \( n = 5 \)) for all complex carbohydrates and sweeteners measured. No participant was more sensitive and experienced high intensity or less sensitive and experienced low intensity to all complex carbohydrates and sweeteners tested across both measures (DTs and STs).

When participants were further stratified into tertile groups (DTs) according to the four taste primaries (sour, salty, bitter, umami) and complex carbohydrates, we observed that two participants \( (n = 2) \) were more sensitive towards all four-taste primaries. Similarly, some participants experienced high intensity \( (n = 4) \) or low intensity \( (n = 4) \) when stratified into tertile groups (STs) according to the four taste primaries (sour, salty, bitter, umami) and complex carbohydrates. No participant was more sensitive and experienced high intensity or less sensitive and experienced low intensity towards all four taste qualities and complex carbohydrates across both measures (DTs and STs).

5.5 Discussion

Our data support the hypothesis that complex carbohydrates (maltodextrin, oligofructose) can be sensed in the oral cavity over a range of concentrations by human participants. Furthermore, our data predicate that oral sensitivity to complex carbohydrates (maltodextrin, oligofructose) is not related to DTs of sweeteners (and other prototypical tastants) but there is overlap with perceived sweetener intensities.
The prevailing understanding at present is that the human taste system is now widely accepted to include five basic tastes (sweet, sour, bitter, salty, and umami taste), and fat taste being accepted by a few. (55-65) Nevertheless, fat taste does not appear to have the same perceptual salience as the other five basic taste qualities. (116) Rather, the reported ‘taste’ resembling effects from orally perceivable fatty acids only appear to be true at a DT level (lowest level at which a difference can be detected) as fatty acids do not stimulate suprathreshold taste intensity perception like the other five primary taste qualities. (55, 309) Furthermore, intensity perception for long chain fatty acids is controversial as intensity may be a function of irritation, smell, or any textural sensation. (62) In order for oral perception of complex carbohydrates to be classified as a taste component, certain criteria that have been proposed previously should be met. (61, 223) These criteria comprise the following: 1) provides an adaptive (evolutionary) advantage; 2) is elicited by a unique class of chemicals; 3) has an independent transduction mechanism; 4) signals are detected through gustatory nerves that are processed in the gustatory cortex; 5) is perceptible and has a unique sensation that does not overlap with any other prototypical taste qualities; and 6) raises a behavioural and/or physiological reaction. (61, 223, 224) In the following paragraphs, the discussion will consider the evidence supporting complex carbohydrate as a “taste” component related to each of these criteria.

In regards to complex carbohydrates, the evidence outlined in the present study provides support for two of the stipulated criteria for a taste primary, i.e. is elicited by a unique class of chemicals and perceptual independence (perceptual independence with sweet taste is at DT only, but overlap with sweetness at intensity). At present, our data provide evidence that complex carbohydrates (oligosaccharides: maltodextrin, oligofructose) are perceptible and there were no robust correlations observed between
the four basic taste primaries (salty, sour, bitter, and umami tastes; both DTs and STs) and DTs and STs of both complex carbohydrates (maltodextrin and oligofructose). For sweet taste, DTs of the complex carbohydrates (maltodextrin, oligofructose) and all of the sweeteners were correlated. However, there were moderate correlations between the STs of the complex carbohydrates and sweeteners. In light of our methodological approach, that is: a) participants were asked not to swallow any samples during testing, mouth rinsing with deionised water between tasting samples, and the use of nose clips to eliminate any orthonasal and retronasal olfaction cues; b) use of red lights to reduce any perceptual differences due to colour (visual) of samples; c) repeated testing of up to seven times per complex carbohydrate and good test-retest reliability of complex carbohydrates; d) a wide range of concentrations used starting from low concentration levels; and e) solutions were prepared fresh on the day, we are confident that the DTs and STs reported were unique to oral taste sensitivity to complex carbohydrates, and not based on additional orosensory cues such as olfaction and visual. However, the most challenging potential confound is with texture/viscosity, especially at higher concentration levels. Although we observed that some participants were able to consistently differentiate complex carbohydrate solutions from water at the lowest concentration levels tested (0.04% w/v), still, the evidence is not conclusive that the DTs and STs reported were not due to additional textural cues. Therefore, while not diminishing the prospect that oral complex carbohydrate sensitivity could be due to textural differences, the present finding suggests that complex carbohydrates are perceptible in the oral cavity and have a distinct oral sensation that does not overlap with any primary taste qualities (perceptual independence with sweet taste is at DT only, whereas there is overlap with sweetness at intensity). These findings are consistent with Lapis et al. (18), where the STs of maltodextrin (DE5 and 10) were not
significantly correlated to sodium chloride (salty taste), glucose (sweet taste) and sucrose (sweet taste). Furthermore, the present finding refutes the historical assumption that complex carbohydrates are tasteless to the human palate system. (6, 330-332)

One obstacle to acceptance of complex carbohydrate as a taste quality has been identification of potential pathways and or receptor(s) for oral complex carbohydrate perception. At present, it is widely accepted that the sweet taste receptors are the only carbohydrate sensing receptors in the oral cavity. The primary sweet sensor, the sweet taste receptor consists of two heterodimer G-protein coupled receptors, the T1R2-T1R3. (123) The T1R2 and T1R3 dimers entail a large extracellular area (i.e. Venus flytrap domain), which is connected to the transmembrane via a cysteine-rich domain. (111) It has been suggested that the cysteine-rich domains activate sweet proteins, whereas, the Venus flytrap domain of T1R2 targets a large variety of sweet substances (caloric sweeteners and most of the NNS) and the Venus flytrap domain of T1R3 targets other NNS, such as cyclamate and sweet receptor blocker, lactisole. (95, 111) A significant issue is whether or not complex carbohydrates are detected through the same taste receptor that detects sweetness (i.e. T1R2-T1R3 heterodimer). The present results showed that the discriminability of the caloric sweeteners (glucose, fructose, sucrose, and erythritol) from water were about the same, as were NNS (sucralose and Rebaudioside A). There were also strong correlations between the DTs of the complex carbohydrates (maltodextrin, oligofructose). However, the DTs of the complex carbohydrates and all of the sweeteners were not correlated, highlighting that mechanisms other than the T1R2-T1R3 are responsible for the detection of complex carbohydrates. Considering the concentrations used, it is possible that the participants detected the complex carbohydrates in the maltodextrin samples instead of the free
sugars. The current data is consistent with the previous psychophysical studies where participants were found to be able to perceive complex carbohydrates (glucose polymer, glucose oligomers), and the sensitivity to simple sugar (glucose) was independent of that to complex carbohydrates. (18, 20) In the study by Lapis et al. (20), it was found that humans ($n = 25$) were able to discriminate complex carbohydrate solutions (glucose oligomers) from water even when the sweet taste receptor (T1R2-T1R3 heterodimer) was inhibited by lactisole treatment—a sweet taste blocker known to bind to a pocket in the transmembrane region of the T1R3 and thus inhibits the sweet taste perception of sugars, proteins and NNS. (109) Remarkably, although Lapis et al. (18) observed large individual variances between participants in terms of $\alpha$-amylase activity, taste responsiveness to maltodextrin (DE 20, 10, and 5) was not significantly different between groups of participants with high $\alpha$-amylase activity and low $\alpha$-amylase activity.

The present study is also in line with the results of animal studies in which knockout mice missing functional genes for both components of the sweet taste receptor (heterodimer of T1R2 and T1R3) show no genetic, electrophysiological, and behavioural reactions to simple sugars (glucose, fructose, or sucrose) but respond normally to complex carbohydrates. (15, 228-232, 333) Besides, acceptability of complex carbohydrate (maltodextrin) was found to be unaccounted for by the small amount of free sugars (~0.05-2.88% w/v glucose and maltose) contained in maltodextrin, but rather, rodents appear to be highly attracted to the complex carbohydrate (maltooligosaccharide) itself. (2, 233, 234) Explicitly, rats ingested more of the maltodextrin solutions when tested at lower concentrations (1-4% w/v, total sugars in samples ~ 0.05-0.36% w/v), but decreased intake at higher concentrations (8-32% w/v, total sugars in samples ~ 0.72-2.88% w/v). Together, these findings raise the
potential existence of an unidentified complex carbohydrate taste receptor in humans that responds to complex carbohydrates independently of those of sweet tastants (at lower concentration levels). (8)

Interestingly, at present, there were moderate correlations between the STs of complex carbohydrates and sweeteners. Potential explanation for this is that a novel receptor might still be involved in the transduction mechanism used to detect complex carbohydrates, but only for the detection range. At the perceptual range, the perception of complex carbohydrates (maltodextrin) could be partly mediated by the T1R-independent sweet sensing pathways in addition to the putative complex carbohydrate detection receptor [see discussion in Lapis et al. (18, 20)]. It is also possible that the taste cell expressed enzymes such as salivary α-amylase, sucrose-isomaltase, and maltase-glucoamylase enzymes may locally break down dietary oligosaccharides, disaccharides, and starch hydrolysis products into monosaccharides. Thus, the monosaccharides and free sugars in complex carbohydrates (maltodextrin) may combine to activate the T1R2-T1R3 sweet taste receptor and/or T1R-independent sweet pathway in taste receptor cells, which could explain the commonality seen with sweet taste in the perceptual range. However, at the detection range, maltose/maltotriose has a weak intensity of sweetness and the amount of free sugars in complex carbohydrates (maltodextrin) may be too low to activate the salivary α-amylase enzymes. Thus, this explanation may potentially explain why we only observed commonality with the sweet taste mechanism for the perceived intensity range, but not at the detection ranges. Given that oral expressed enzymes would be ineffective in hydrolysing oligofructose, it is unknown at this stage why commonalities were observed between oligofructose and the sweeteners measured.
Chapter 5 – Study 2(a) Evidence Supporting Oral Sensitivity to Complex Carbohydrates Independent of Sweet Taste Sensitivity in Humans

The finding that complex carbohydrates, maltodextrin and oligofructose, were strongly correlated with each other suggests some sort of similarity between both complex carbohydrates in terms of transduction pathways. We are uncertain why these similarities were observed given that oligofructose has been described as being thirty percent as sweet as sucrose and is used as low-calorie sweetener in foods. (334, 335) Furthermore, the chemical structure is different between both of these complex carbohydrates and there does not appear to be any published studies showing that oligofructose has a preferred or maltodextrin-like taste to rodents. However, in studies investigating the effects of oligofructose on appetite profiles, maltodextrin was used as placebo supplements as they have been suggested to have a similar appearance and oral sensation as oligofructose. (272, 273, 275) It is also possible that similarities were observed between both complex carbohydrates in this study as they have a similar texture or mouthfeel, thus seeing commonalities between them.

There was large inter-individual variation in oral complex carbohydrate perception, and individuals may be classified as more or less sensitive to complex carbohydrates based on their sensitivity towards complex carbohydrates. For example, the concentration required to reach DT for maltodextrin varied 158 fold across the sample population. There was also large individual difference in perceived complex carbohydrate intensity. For example, the same maltodextrin sample (20% w/v) was rated 2.5 gLMS by one participant but 32.9 gLMS by another. Inter-individual differences or variability in taste function has also been previously observed for other taste qualities such as sweet. (70, 157, 158, 294) However, it is possible that large inter-individual differences in oral complex carbohydrate sensitivity were observed because of individual differences in AMY1 gene copy number and salivary α-amylase levels (336) but not taste. In this study, individuals with lower salivary amylase levels
reported slower and significantly lesser decrease in perceived oral starch viscosity (oral viscosity thinning) in comparison to individuals with higher salivary amylase activity. (336)

The current evidence from animal studies and human exercise studies provides support for the remaining stipulated criteria for oral complex carbohydrate sensitivity as a taste component (i.e. criteria 1, 4, and 6). Considering the evolutionary advantages of our taste system, it could be argued that the physiological regulation and functional significance of sensing low amounts of complex carbohydrate is beneficial to the survival of human beings, especially during times when foods are scarce as complex carbohydrates represent a major source of energy for body functioning. (67) The adaptive advantage of complex carbohydrate sensing in the oral cavity is supported with the behavioural evidence from animal studies where rodents prefer complex carbohydrate solutions to solutions containing simple sugars, especially at low equimolar concentrations. (6, 17, 242) In addition, Sclafani and Mann (14) reported that rats prefer maltodextrin to sugars at low molar concentrations in three minute two-bottle choice tests, which limits post-ingestive influences and learning. In a recent study by Poole et al. (337) investigating the phenotypic differences among eight inbred strains of mouse, strain variation in complex carbohydrate (maltodextrin) perception that is distinct from variation in sweet (sucrose) perception has been observed. More recent physiological evidence from exercise science found that exercise performance significantly improved after participants rinsed their mouth with solutions containing complex carbohydrate (maltodextrin) compared to NNS control solutions. Similarly, these findings were also replicated by other exercise scientists. (245-247, 249-251, 338-340) Additionally, Chambers et al. (244) further investigated the corticol response to oral maltodextrin and glucose solutions, revealing a similar pattern of brain
activation in response to both solutions, including brain areas believed to be involved in the reward system (i.e. activates brain reward centres in orbitofrontal cortex and striatum similar to oral glucose, which were unresponsive to NNS). Together, these findings provide strong behavioural and physiological evidence that there may be taste transduction pathways that respond to complex carbohydrate independently of those for sweet taste. (251) Supporting one of the six criteria for oral perception of complex carbohydrates to be classified as a taste component, one study by Vigorito et al. (241) provided evidence that there is some specialization of function within the rat’s peripheral gustatory system in response to complex carbohydrates. The results of this study revealed that selective gustatory nerve transection of the chorda tympani nerve, glossopharyngeal nerve, greater superficial petrosal nerve, and the pharyngeal branch of the vagus nerve differentially altered the intake of sucrose and maltodextrin solutions. (241) Interestingly, gustatory denervation of all four gustatory nerves (chorda tympani, glossopharyngeal nerve, greater superficial petrosal nerve, and chorda tympani nerve) in rats reduced their intake of both sucrose and maltodextrin solutions by the same degree. (241) These results indicate that while the intake of sucrose and maltodextrin appeared to be facilitated to the same level by the gustatory system, the pathways involved appear to vary. (2, 241)

The evidence outlined in the present study provides support for each of the proposed criteria for a taste component. However, due to the limited studies conducted in humans, the evidence supporting most of the criteria is not conclusive and thus warrants further investigation. There are some limitations that need to be taken into account when considering the results. It is important to acknowledge that this study does not control for salivary α-amylase during sample testing. Salivary amylase has been shown to hydrolyse α-1, 4 glycosidic bonds once mixed with complex
carbohydrates, resulting in changes in texture. Thus, we were unable to rule out the possibility that participants experienced differences in oral complex carbohydrate sensitivity due to differences in texture instead of the “taste” component. Therefore, more evidence from tribology studies is required to ensure that the DTs and STs reported were not due to textural cues.
Chapter 5 – Study 2(a) Evidence Supporting Oral Sensitivity to Complex Carbohydrates Independent of Sweet Taste Sensitivity in Humans

5.5 Conclusion

Contrary to the previous understandings of the human taste system where complex carbohydrates have long been assumed to be tasteless to the human palate, our data highlight that complex carbohydrates (maltodextrin, oligofructose) are perceptible in the oral cavity and have a distinct oral sensation that does not overlap with any primary taste qualities. Additionally, our data indicate that oral sensitivity to complex carbohydrate is not related to a range of sweeteners at low concentration levels (DTs). The findings are consistent with the proposition of an independent mechanism for complex carbohydrates, but only for lower concentration levels. At the perceptual range, it is possible that the perception of complex carbohydrates may be partly mediated by the T1R-independent sweet sensing pathways in addition to the putative complex carbohydrate detection receptor. Another possibility is that the taste cell expressed enzymes such as the salivary α-amylase enzymes may locally break down dietary oligosaccharides, disaccharides, and starch hydrolysis products into monosaccharides. Thus, the monosaccharides and free sugars in complex carbohydrates (maltodextrin) combine to activate the T1R2-T1R3 sweet taste receptor and/or T1R-independent sweet pathway in taste receptor cells thereby showing the commonality with sweet taste in the perceptual range. However, it is unknown at this stage why commonalities were observed between oligofructose and the sweeteners measured.
**Chapter Six: Study 2(b) The Associations between Oral Complex Carbohydrate Sensitivity, Body Composition, and Dietary Intake in Adults**

1*This study is currently under review at Journal of Nutrition as ‘The Associations between Oral Complex Carbohydrate Sensitivity, Anthropometry, and Dietary Intake in Adults’.*

### 6.1 Introduction

The increasing worldwide prevalence of nutrition-related chronic illness such as obesity requires a greater understanding of the drivers of food intake. Increased energy intake is thought to be one of the major contributors to the global rise of being overweight and obese. (31, 303) Carbohydrates in the form of simple and complex carbohydrates represent a major source of energy in our diet. For example, the estimated Acceptable Macronutrient Distribution Ranges (AMDR) related to reduced risk of chronic disease are 45-65% of total energy intake from carbohydrate, 20-35% from fat, and 15-25% from protein. (367)

The function of complex carbohydrates in our diet has considerably changed since the introduction of agriculture around eight to ten thousand years ago. (306) In comparison to other animals, the independent origin of salivary amylase to digest complex carbohydrates in the oral cavity in rodents and primates suggests that there has been strong evolutionary selection for amylase in saliva (See review by Meisler and Ting (341) on evolutionary history of the human amylase genes). Bearing in mind that the physiological function of many nutrient sensors within the oral cavity is to sense the nutritious or toxic qualities in foods, (67, 306) logically, components of
complex carbohydrates would be detected in the mouth, similar to other nutritive components such as proteins (e.g. monosodium glutamate (MSG)), and fats (e.g. oleic acid). (55-59, 61-64, 150, 342) However, as opposed to simple carbohydrates, complex carbohydrates have long been assumed invisible to the human palate, (6, 330) and as such have been used as tasteless caloric ingredients in flavour-nutrient conditioning studies (e.g. de Araujo et al. (331), Yeomans et al. (343) and Yeomans (332)). Conversely, current evidence suggests that humans may perceive complex carbohydrates and that sensitivity to simple carbohydrates is independent of that to complex carbohydrates (i.e. perceived intensities for simple carbohydrates such as glucose and sucrose were significantly correlated with each other, but not with complex carbohydrates). (18, 19) Consistent with these findings, recent work from our laboratory (Chapter 5) has suggested that complex carbohydrates could be consistently sensed in the oral cavity over a range of concentrations. Furthermore, our data also predicate that oral sensitivity to complex carbohydrates is not related to sensitivity to a range of sweeteners and prototypical tastants, suggesting that humans detect and perceive complex carbohydrates as qualitatively different from the five basic taste qualities (Chapter 5). However, it is important to note that perceptual independence with sweet taste is at detection threshold only, but overlap with sweetness at intensity (Chapter 5).

Individual differences in taste sensitivity and the role of taste in promoting intake of specific foods or ingredients associated with obesity has been a long-investigated area of research but with mixed findings. (55, 56, 63, 158, 179, 181-184) However, in regards to sweet taste, the relationship between environmental influences such as habitual diet and sweet taste function is complicated, where most data has shown no link between sweet taste function, BMI, and dietary intake. (158, 189-196,
Furthermore, in Chapter 4, results have shown that sweet taste function is not associated with body composition and sweetness intensity in comparison to threshold measures are the most appropriate measure when assessing links between sweet taste and food consumption. Whether or not habituation occurs for complex carbohydrates remains unclear, and is of particular interest in order to understand why some individuals consume more energy.

6.2 Aims, Hypotheses, and Terminologies

6.2.1 Aims

The aim of this study was to investigate associations between oral complex carbohydrate sensitivity, body composition, and dietary intake among adults.

6.2.2 Hypotheses

- Oral suprathreshold intensity perception for complex carbohydrate will be negatively associated with body composition, and dietary intake; but detection threshold for complex carbohydrate will not be associated with body composition, and dietary intake.

6.2.3 Terminologies

Please refer to Section 5.2.3.
6.3 Subjects, Materials, and methods

6.3.1 Study Design

This study comprised a total of 15 laboratory-based sessions in which demographic (age, sex), anthropometric (height, weight, waist circumference) and dietary data (4-day diet diary, food frequency questionnaire) were collected in addition to data on two measures of taste perception routinely used in chemosensory research: (1) detection threshold (DT) and (2) suprathreshold intensity rating (ST). These measures were described in Chapter 5. Participants in the present study were part of a larger study focusing on the psychophysics of complex carbohydrate sensitivity (344) and sweet taste function. (270, 299) In this study, DTs and STs were determined for all participants for each of two complex carbohydrates, six sweeteners and prototypical stimuli for sour, salty, umami, and bitter (Chapter 5). Filtered deionised water was used as an oral rinsing agent. Participants were instructed to rinse their mouths with filtered deionised water for five seconds before beginning each task and between each sample set. To eliminate any visual and olfactory input, all testing sessions were conducted under red lighting, and participants were asked to wear nose clips during testing. All solutions were served at room temperature, with a three-digit code allocated to each sample.

6.3.2 Subjects

Please refer to Section 5.3.2 for subject details.
6.3.3 Participant Training

Please refer to Section 5.3.3 for details on participant training.

6.3.4 Stimuli

Maltodextrin and oligofructose were used to investigate oral complex carbohydrate sensitivity (for details of stimuli see Chapter 5). There were a total of 2.8g/100g (2.8% w/w) of free sugars for the maltodextrin (Glucose: 0.8% w/w) and 3.0g/100g (3.0% w/w) of free sugars for the oligofructose (Fructose: 1.4% w/w) used in this study (see Section 5.3.5 for composition of the complex carbohydrates used in the present study).

6.3.5 Detection Threshold Determination and Suprathreshold Intensity Ratings for Oral Sensitivity to Complex Carbohydrates

The method for this section has previously been described in Section 5.3.6 and Section 5.3.8.

6.3.6 Body Composition

As detailed in Chapter 2, all participants were asked to remove their shoes and any heavy clothing to ensure accurate measurements. All body composition measurements were measured first thing during the initial and final visits after a 1-hour fast (food only). Participants’ body weight was measured to the nearest 0.1 kg using a segmental body composition analyser (TBF-300A) (Tanita Corporation, Tokyo, Japan). Participants’ height was measured to the nearest 0.1 cm using a portable stadiometer (Seca213) (Seca, Hamburg, Germany). All measurements were
repeated twice to ensure accuracy. Averages of measurements for height and weight were used to calculate BMI (weight in kg/m²) and determine weight status \( (i.e. \) normal weight or overweight/obese). Weight status was defined under World Health Organisation BMI classification. (277) Using methods outlined by the Australian Heart Foundation (278), waist circumference was also measured. Waist circumference was measured twice to the nearest 0.1 cm using an ergonomic circumference measurement tape (Seca201) (Seca, Hamburg, Germany). An average of waist circumference measurements was calculated and used for analysis. Waist circumference cut-off points \( (i.e. \) lower risk of metabolic complications or increased risk of metabolic complications (> 94cm males, > 80cm females)\) were defined under the World Health Organization recommended cut-off points. (279)

6.3.7 Dietary Intake

The Dietary Questionnaire for Epidemiology Studies Version 2 (DQESV2), (280) a validated version of the Food Frequency Questionnaire (FFQ) (281, 282) developed by Cancer Council Victoria, was used to measure each participant’s habitual pattern of food intake (detailed in Chapter 2). Participants were required to indicate, on average, how many times in the previous year they consumed a number of food and beverage items (74 items) across four categories \( (i.e. \) 1) cereal foods; sweets and snacks; 2) dairy products, meats and fish; 3) fruit; and 4) vegetables) with 10 frequency response options ranging from ‘never’ to ‘3 or more times per day’. They were also required to indicate the portion size that they normally consumed. Participants were asked to complete the FFQ within a month from their first visit. Using software based on the Australian nutrient composition database NUTTAB95, (283) analysis was
carried out by the Cancer Council Victoria, Australia to assess daily energy and macronutrient intakes.

In addition to the FFQ, participants were asked to complete a 4-day diet diary (3 weekdays, 1 weekend day within a 7-day period) within a month from their first visit, in which they recorded all of the foods and beverages they consumed. Participants were asked to, where possible, measure their foods using measurement cups, spoons or common serving sizes (e.g. one large egg) or to weigh their foods using kitchen scales at home. They were also asked to be as specific as possible, including reporting the type (e.g. skim milk or full fat milk) and brand of food consumed, the cooking methods used (e.g. fried, baked, or steamed) or whether fat was added when cooking (e.g. food cooked in butter or cooking oil). If the food consumed was from a recipe, the participants were asked to include the recipe with the record and to state how much of it they consumed (e.g. a quarter of a recipe). Participants were also given an example of a 1-day diet diary record as a guide to complete the diet diaries. Diet diaries were analysed using FoodWorks 8 (Xyris Software, Highgate Hill, Queensland, Australia). Mean energy intake (kilojoules; kJ) and macronutrient distribution (% energy from fat, protein, and carbohydrate), and the type of carbohydrate (% energy from starch, sugar, fibre) were quantified using the Australian nutrient composition database AUSNUT2011-2013 (286) and compliance checked. Two dietary assessment methods were used to assess dietary intake as they measure two different periods of time (i.e. a year and 4 days), thus would provide a better picture of an individual’s dietary intake.

6.3.8 Statistical analysis
Statistical analysis was performed using IBM SPSS statistical software version 23.0 (SPSS, Chicago, IL, USA). Data are presented as means with standard errors of the mean (SEM). Descriptive statistics were employed to describe demographic information, complex carbohydrate DTs and STs, and dietary intake. The DTs and STs were determined as the arithmetic mean of the repeated measures. For STs, the geometric mean score of the four ratings (weak, medium, medium-strong, and strong) was calculated. Over- and under-reporters for dietary intake were checked for out of range values for energy intake and cases with outlying values [energy intake more than 2 standard deviations (SD) above/below the mean energy intake] were removed from further dietary analyses. However for BMI and waist circumference, all participants were included in the analyses.

Independent t-tests were used to analyse differences in terms of oral complex carbohydrate sensitivity (DTs and STs for both complex carbohydrates) between gender groups. Pearson’s product-moment correlations were also conducted to analyse the relationships between oral complex carbohydrate sensitivity (DTs and STs for both complex carbohydrates) and BMI, waist circumference, and dietary intake. DT for each complex carbohydrate was treated as a grouping variable (tertiles) with participants categorised as more sensitive (1/3), normal sensitive (2/3), and less sensitive (3/3) to explore differences between continuous (waist circumference, BMI, habitual energy intake, and macronutrient intakes) variables. ST for each complex carbohydrate was treated as a grouping variable (tertiles) with participants categorised as those who experienced low intensity (1/3), moderate intensity (2/3), and high intensity (3/3) to explore differences between continuous (waist circumference, BMI, habitual energy intake, and macronutrient intakes) variables. DTs and STs for both complex carbohydrates were grouped into tertiles to allow comparison of most and
least sensitive groups or groups who experienced low and high intensity (*i.e.* four sets of tertiles were determined: one for DT for each complex carbohydrate and one for ST for each complex carbohydrate). (299) Independent *t*-tests were used to detect differences in habitual energy intake, BMI, and waist circumference between more sensitive and less sensitive participants or those who experienced low and high intensity (lower and higher tertile groups). (299) Independent *t*-tests were used to detect differences in diet between more sensitive and less sensitive participants or those who experienced low and high intensity, with macronutrient composition (*i.e.* percent dietary energy from starch, fibre, sugar, and carbohydrate) as a dependent variable and oral complex carbohydrate sensitivity (DTs and STs for both complex carbohydrates) as the independent variable. (299) Independent *t*-tests were used to analyse differences in terms of dietary intake between weight status categories and waist circumference risk groups. Pearson’s product-moment correlations were also conducted to analyse the relationships between dietary assessment tools (*i.e.* dietary intake measured using the 4-day diet diaries and FFQs). Significance was accepted at *P* < 0.05.
6.4 Results

6.4.1 Participants

Sixteen male participants [age 26.2 ± 0.4 years (range 24.0 – 30.0 years), BMI 25.2 ± 0.9 kg/m² (range 18.9 – 30.0 kg/m²), waist circumference 89.0 ± 3.0 cm (range 73.0 – 106.0 cm)] and 18 female participants [age 29.4 ± 2.1 years (range 24.0 – 55.0 years), BMI 24.3 ± 0.8 kg/m² (range 20.0 – 29.6 kg/m²), waist circumference 78.2 ± 1.5 cm (range 68.0 – 85.5 cm] were recruited. Out of the total 34 participants, 18 were classified as overweight/obese [7 male, 11 female, BMI 27.4 ± 0.5 kg/m² (range 25.2–30.0 kg/m²), waist circumference 88.8 ± 2.2 cm (range 75.2 – 106.0 cm)]. There were no significant differences in BMI or age between male and female participants (all \( P > 0.05 \)). However, female participants had significantly smaller waist circumferences in comparison to male participants (\( P < 0.01 \)) (see Table 6.1 for baseline characteristics of study participants).
Table 6.1 *Baseline characteristics of study participants (Mean values and standard errors).*

<table>
<thead>
<tr>
<th></th>
<th>All (n = 34)</th>
<th>Normal Weight (n = 16)</th>
<th>Overweight/Obese (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
</tr>
<tr>
<td>Age (years)</td>
<td>27.9</td>
<td>1.2</td>
<td>26.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>166.8</td>
<td>1.9</td>
<td>166.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69.2</td>
<td>2.6</td>
<td>59.9</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.7</td>
<td>0.6</td>
<td>21.6</td>
</tr>
<tr>
<td>BMI range (kg/m²)</td>
<td>18.9-30.0</td>
<td>18.9-24.9</td>
<td>25.2-30.0</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>83.3</td>
<td>1.8</td>
<td>77.2</td>
</tr>
<tr>
<td>Waist circumference range (cm)</td>
<td>68.0-106.0</td>
<td>68.0-95.7</td>
<td>75.2-106.0</td>
</tr>
<tr>
<td>Lower risk group (frequency)²</td>
<td>16</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>Higher risk group (frequency)²</td>
<td>18</td>
<td>2</td>
<td>16</td>
</tr>
</tbody>
</table>

¹Normal weight, BMI = 18.5-24.9 kg/m²; overweight, BMI = 25-29.9kg/m²; obese, BMI ≥ 30 kg/m². (277) ²Waist circumference group cut-off points: Lower risk of metabolic complications (≤ 94cm males, ≤ 80cm females) or increased risk of metabolic complications (> 94cm males, > 80cm females). (279)
6.4.2 Oral Complex Carbohydrate Sensitivity of Maltodextrin and Oligofructose

There were no significant differences in oral complex carbohydrate sensitivity (DTs and STs for both complex carbohydrates) between male and female participants (all $P > 0.05$); therefore, the data are presented together. The mean DTs and STs, standard error of means, and range for both complex carbohydrates are presented in Table 6.2.

### Table 6.2 Detection thresholds (% w/v) and mean intensity ratings (gLMS) for complex carbohydrates presented as mean, standard error of mean, and range.

<table>
<thead>
<tr>
<th></th>
<th>Detection Threshold ($n = 34$)</th>
<th>Mean Intensity Rating ($n = 34$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM Range</td>
<td>Mean ± SEM Range</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>1.7 ± 0.3 0.04-6.3</td>
<td>11.5 ± 1.5 0.8-31.6</td>
</tr>
<tr>
<td>Oligofructose</td>
<td>1.8 ± 0.4 0.04-7.7</td>
<td>11.5 ± 1.4 1.9-31.1</td>
</tr>
</tbody>
</table>

6.4.3 Oral Complex Carbohydrate Sensitivity and Body Composition

No significant correlations were identified between any measures of oral complex carbohydrate sensitivity (DTs and STs) and BMI for either complex carbohydrate (all $P > 0.05$). Similarly, when grouped into tertiles according to their oral complex carbohydrate sensitivity (DTs and STs for both complex carbohydrates), Tukey post hoc analyses revealed no significant differences in BMI between the more sensitive and less sensitive participants or participants who experienced high and low intensity for either complex carbohydrate (all $P > 0.05$) (Figure 6.1a and Figure 6.1b). When stratified into weight status, there were no significant differences in any measure of oral complex carbohydrate sensitivity (DTs and STs for both complex carbohydrates) between normal weight and overweight/obese participants (all $P >$
However, significant correlations were observed between oral complex carbohydrate sensitivity and waist circumference for maltodextrin DT ($r = -0.38$, $P < 0.05$) and maltodextrin ST ($r = 0.48$, $P < 0.05$); as well as oligofructose ST ($r = 0.42$, $P < 0.05$), but not oligofructose DT ($r = -0.30$, $P = 0.08$). Likewise, when grouped into tertiles according to their oral complex carbohydrate sensitivity (DTs and STs for both complex carbohydrates), Tukey post hoc analyses revealed significant differences in waist circumference between the more sensitive and less sensitive participants for maltodextrin (Figure 6.1c). There were also significant differences in waist circumference between participants who experienced high and low intensity for either complex carbohydrate (Figure 6.1d). Participants who were more sensitive towards maltodextrin (DT) had bigger waist measurements (mean = 91.4cm) in comparison to less sensitive participants (mean = 80.5cm) ($P < 0.05$). For intensity ratings, participants who experienced high intensity towards both complex carbohydrates (maltodextrin and oligofructose), had bigger waist measurements (maltodextrin: mean = 89.3 cm; oligofructose: mean = 88.2 cm) in comparison to those who experienced low intensity (maltodextrin: mean = 75.5cm; oligofructose: mean = 76.6 cm) (all $P < 0.01$). Similar relationships were observed when stratified into waist circumference risk groups; there were significant differences in STs of complex carbohydrates between participants with lower waist circumference (mean ST maltodextrin: 6.1 gLMS, oligofructose: 6.7 gLMS) and higher waist circumference (mean ST maltodextrin: 16.3 gLMS, oligofructose: 15.8 gLMS) (all $P < 0.01$). There were, however, no significant differences for DT complex carbohydrate measurements when stratified into waist circumference risk groups ($P > 0.05$).
Figure 6.1 (A-B) BMI mean and standard errors between more sensitive/less sensitive participants and those who experienced high intensity/low intensity. (C-D) Waist circumference mean and standard errors between more sensitive/less sensitive participants and those who experienced high intensity/low intensity. *$P < 0.05$, **$P < 0.01$. 
6.4.4 Oral Complex Carbohydrate Sensitivity and Energy and Macronutrient Intakes

One participant was identified as an under-reporter of energy intake [energy intake more than 2 SD (1485 kJ) below the mean energy intake (7656 kJ)] using the diet diary records, whereas two participants were identified as over-reporters of energy intake [energy intake more than 2 SD (2381 kJ) above the mean energy intake (7204 kJ)] via the FFQ measure. Means (± SEM) for energy and macronutrient (as percentages of energy intake) intakes are presented in Table 6.3.

<table>
<thead>
<tr>
<th></th>
<th>Diet Diaries (n = 33)</th>
<th>FFQ (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total energy (kJ)</td>
<td>7757.9 ± 244.8</td>
<td>7888.8 ± 278.4</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>34.6 ± 0.9</td>
<td>37.7 ± 0.6</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>20.8 ± 0.9</td>
<td>20.3 ± 0.6</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>41.9 ± 1.4</td>
<td>42.5 ± 0.9</td>
</tr>
<tr>
<td>Sugar (%)</td>
<td>13.1 ± 0.9</td>
<td>16.3 ± 1.2</td>
</tr>
<tr>
<td>Starch (%)</td>
<td>29.6 ± 1.3</td>
<td>26.0 ± 1.3</td>
</tr>
<tr>
<td>Fibre (%)</td>
<td>2.1 ± 0.1</td>
<td>2.1 ± 0.1</td>
</tr>
</tbody>
</table>

Using the diet diaries as a measurement for dietary intake, no robust associations between measures of oral complex carbohydrate sensitivity (DTs and STs), percent energy from fat, protein, carbohydrate, sugar, starch, or fibre were observed. However, a significant correlation was observed between mean total energy intake and maltodextrin ST ($r = 0.39$, $P < 0.05$). When grouped into tertiles, Tukey post hoc analyses revealed significant differences between those who experienced low and high intensity in terms of mean total energy consumed per day (Figure 6.2b). Participants who experienced high intensity towards maltodextrin solutions consumed significantly more energy per day (mean = 7968kJ) in comparison to those who experienced low intensity (mean = 6693kJ) ($P < 0.01$). No significant correlations
were identified for mean total energy intake and maltodextrin DT \((r = 0.04, P = 0.84)\), oligofructose DT \((r = 0.14, P = 0.45)\), or oligofructose intensity ratings \((r = 0.33, P = 0.06)\).

Using the FFQ as a measurement for dietary intake, no significant correlations between DT with mean total energy intake, percent energy from fat, protein, carbohydrate, sugar, starch, or fibre were observed (all \(P > 0.05\)). However, when grouped into tertiles, Tukey post hoc analyses revealed significant differences between more sensitive and less sensitive participants (maltodextrin DT) in terms of mean total energy consumed per day (Figure 6.2a). Participants who are more sensitive towards maltodextrin (DT) consumed significantly more energy per day (8954 kJ) in comparison to less sensitive participants (7747 kJ) \((P < 0.05)\). For STs, significant correlations were observed between mean total energy intake and maltodextrin ST \((r = 0.37, P < 0.05)\). When grouped into tertiles, participants who experienced high intensity towards maltodextrin solutions consumed significantly more energy per day (mean = 8149 kJ) compared to those who experienced low intensity (mean = 6767 kJ) \((P < 0.05; \text{Figure 6.2b})\). No significant correlations were identified for mean total energy intake and oligofructose ST \((r = 0.29, P = 0.09)\). Interestingly, significant correlations were observed between percent energy from starch and STs for both complex carbohydrates (maltodextrin: \(r = 0.40, P < 0.05\); oligofructose: \(r = 0.36, P < 0.05\)). When grouped into tertiles, participants who experienced high intensity towards both maltodextrin and oligofructose, consumed significantly more energy from starch (maltodextrin: mean = 29.1%, oligofructose: mean = 29.8%) in comparison to those who experienced low intensity (maltodextrin: mean = 20.9%, oligofructose: mean = 22.2%) \((P < 0.05; \text{Figure 6.2d})\). No correlations were observed between STs for complex carbohydrates and percent energy from fat, protein, carbohydrate, sugar, or
fibre (all $P > 0.05$). No robust differences were observed between male and female participants in terms of the associations between measures of oral complex carbohydrate sensitivity (DTs and STs) and energy intake.

### 6.4.5 Associations between Body Composition Measurements and Dietary Intake

When stratified into weight status categories, there were no significant differences in dietary intake between normal weight and overweight/obese participants (all $P > 0.05$). Similarly, for waist circumference, there were no significant differences in dietary intake between participants with lower risk of metabolic complications and increased risk of metabolic complications (all $P > 0.05$).

### 6.4.6 Correlations between Dietary Assessment Tools

There were significant correlations for total energy intake and percent energy from carbohydrate, sugar, starch, fibre, and protein per day between the 4-day diet diaries and FFQs (all $P < 0.05$). However, for dietary fat, no significant correlations were observed between the two dietary assessment tools ($P > 0.05$).
Figure 6.2 (A-B) Mean energy intake per day and standard errors between more sensitive/less sensitive participants and those who experienced high intensity and low intensity. (C-D) Mean percentage energy from starch consumed per day and standard errors between more sensitive/less sensitive participants and those who experienced high intensity and low intensity. *P < 0.05.
6.5 Discussion

To our knowledge, this was the first study to examine if oral complex carbohydrate sensitivity (DTs and STs for two complex carbohydrates) was related to body composition measurements (BMI, waist circumferences) or dietary intake (energy and macronutrient intakes). Participants who were orally more sensitive/experienced high intensity to complex carbohydrates (maltodextrin and oligofructose, but mostly for maltodextrin) tended to consume more energy and starchy foods per day, and generally had bigger waist circumference measurements than participants who were less sensitive/experienced low intensity to the complex carbohydrates. Taken together, these results reveal a novel association between complex carbohydrate sensing and the consumption of complex carbohydrates, which may influence waist circumference.

Deviations in any or several nutrient receptors are known to influence intake of specific foods or food components related to the nutrient receptor. (253) The effect of habituation of specific nutrient consumption on taste sensitivity to specific compounds has been previously reported in various studies. For example, in a small, well-controlled three-month dietary intervention between a low-sugar diet group and a control group consuming their normal diet, it was reported that the low-sugar diet group rated sweet foods as more intense in the second and third month in comparison to the normal diet group. (116) However, the effect of the dietary intervention quickly returned back to baseline one month post intervention after the low-sugar diet group resumed their normal diet. (116) Hence, we hypothesised that a similar relationship may exist with complex carbohydrate whereby decreased oral complex carbohydrate sensitivity or experiencing low intensity for carbohydrates would be associated with higher dietary starch or energy intake, as the taste system may adapt to high levels of
dietary starch consumption. However, in contrast to dietary sugar, the results from the present study do not support this hypothesis as participants who experienced high intensity for either complex carbohydrate consumed more starch as a percentage of total energy intakes (FFQ data). Participants who experienced high intensity/were more sensitive to maltodextrin also consumed significantly more total energy per day (both diet diary and FFQ data). It could be argued that the physiological regulation and functional significance of sensing low amounts of complex carbohydrates could be an advantage, as complex carbohydrates represent a major source of energy for physiological functions. It has been suggested recently that sweet taste perception or the degree of sweetness in general is not a good proxy for the amount of energy available in a food. (68, 116) Rather, a detection mechanism encouraging consumption of complex carbohydrate independent from sweet taste may be advantageous to provide quantitative information about the energy (glucose) content for physiological functioning. (67, 68) Considering the small amount of free sugars available in the complex carbohydrates, it could be possible for participants to perceive simple carbohydrates instead of the complex carbohydrates in the samples. However, this is unlikely given that some participants were able to detect complex carbohydrates at low concentration levels, but the same participants were only able to detect simple carbohydrates at higher concentrations (e.g. one participant was able to detect maltodextrin at 0.04% w/v (Glucose: 0.0003% w/v, total sugars in maltodextrin: 0.0011% w/v)). (344) These data provide confidence that we are measuring a phenomena that is not triggered by the small amount of simple carbohydrates in the samples. Furthermore, these results could be partly explained by animal models. For example, in a study by Sclafani (16) investigating the effects of carbohydrate type (simple carbohydrate versus complex carbohydrate) on body weight and diet in rats,
no significant differences in total caloric intake, weight gain, percent body fat, or basal insulin level were observed between groups of rats fed either simple carbohydrate or complex carbohydrate solutions in addition to chow and water for 40 days. However, the complex carbohydrate (maltodextrin) solution group consumed significantly more maltodextrin solution than did the simple carbohydrate group over the 40-day period, (16) suggesting that long-term exposure to complex carbohydrates does not reduce subsequent intake of complex carbohydrates. In another study investigating the preference threshold for complex carbohydrate (maltodextrin) and simple carbohydrate (maltose, sucrose), rats were able to detect maltodextrin in water at a much lower concentration level (25 to 26 times lower) in comparison to maltose and sucrose. (345) Although differences between rats in terms of their sensitivity towards complex carbohydrates were not investigated, in the context of what we found in the present study, it is possible that the more sensitive participants or those who experienced high intensity consumed more energy and starch, as they could sense complex carbohydrates in foods with lower amounts of complex carbohydrates, in comparison to less sensitive participants or those who experienced low intensity (i.e. some participants in the present study could detect complex carbohydrates as low as 0.04% w/v in water). Given complex carbohydrates such as maltodextrin are commonly used in low amounts as a food additive in a wide range of products, more sensitive participants or those who experienced high intensity could find these products as more palatable and thus consume them in higher quantities in comparison to less sensitive participants or those who experienced low intensity. However, we did not measure participants’ liking of foods so we do not know if more and less sensitive participants or those who experienced high and low intensity differ in their liking of starchy foods.
Chapter 6 – Study 2(b) The Associations between Oral Complex Carbohydrate Sensitivity, Body Composition, and Dietary Intake in Adults

The observation that waist circumference differed between the more sensitive and less sensitive groups of participants and those who experienced high and low intensity for all complex carbohydrate measures (except oligofructose DT); and that oral sensitivity towards complex carbohydrates (DTs and STs) was also negatively associated with energy and starch intakes raises the possibility that food intake, may, in part be regulated by both oral sensory and post-digestive nutritive feedback. That is, it is possible that individuals with heightened oral sensitivity responses towards complex carbohydrates may have developed preferences for complex carbohydrate flavours due to post-digestive nutritive cues (conditioned preferences), leading to greater intake of energy and starch, and thus weight gain. (346-348) Post-digestive modulation of complex carbohydrate intake has previously been established in rat models. For example, Sclafani and Nissenbaum (349) observed that rats rapidly develop preferences for flavours associated with intragastric infusions of maltodextrin. These differences were also observed in long-term tests where rats switched their initial preference for sucrose powder over bitter-tasting maltodextrin solutions after continued observation over 24-hours, suggesting that these differences were observed due to the post-digestive nutritive feedbacks (i.e. rate of absorption as influenced by diet form). (2) In relevance to human feeding behaviour, excessive consumption of the liquid form of simple carbohydrate, in particular sugar-sweetened beverages, has been linked to the rising rates of overweight and obesity worldwide due these beverages’ high-energy content, low satiety value, and tendency to lead to incomplete energy compensation in children and adults. (35, 36, 350) However, it is also possible that waist circumference itself could influence oral sensitivity to complex carbohydrate rather than the reverse. For example, high visceral fat depots are associated with
distinct endocrine dysfunction and metabolic profiles, which may possibly influence individual taste perception. (351, 352)

Significant relationships were observed between oral sensitivity towards complex carbohydrate and waist circumference but not BMI measurements. Although BMI measurements (weight distribution) are widely used for the evaluation of obesity both in adults, (353, 354) and children; (355, 356) previous studies have found that in comparison to BMI, abdominal obesity assessed by waist circumference, is a stronger predictor of obesity-related health risk. (357) It is possible that no significant relationships were observed between BMI and oral sensitivity towards complex carbohydrate as the distribution of fat in the body, particularly around the abdominals, might be a better indicator of effect on health status due to greater intake of energy and starch for individuals with heightened oral sensitivity responses towards complex carbohydrates in comparison to body weight. (357) Furthermore, there is also a possibility that the discrepancies in results between waist circumference and BMI measurements could be due to the overlap between waist circumference cut-off points for the normal weight/obese participants. In other words, there were some normal weight participants with waist circumferences above the cut-offs and some overweight/obese participants with waist circumferences below the cut-offs (e.g. one female participant had a waist circumference of 95.7cm, but a BMI of 24.7, and one male participant had a waist circumference of 75.2cm but a BMI of 25.9).

There are some limitations that need to be taken into account when considering the results. First, it is important to acknowledge that perception of tastant solutions in a laboratory setting bears little relevance to actual intake of real food in everyday life. (318) Second, even though many participants were able to consistently differentiate complex carbohydrate solutions from water even at low concentration levels (i.e.
0.04% w/v), more evidence from tribology studies is required to ensure that the DTs and STs reported were not due to textural cues. The approach of categorising the oral complex carbohydrate sensitivity measures into groups of tertiles likely reduces the power of this study. However, we also examined the associations between sensitivity measures, anthropometry, and energy and macronutrient intakes using continuous data. Considering that both approaches generally produced the same outcomes, this gave confidence to the reliability of the results. Finally, there was unequal distribution between males and females in the BMI groups which may have reduced the prospect of seeing any influence of BMI.

6.6 Conclusions

There was large inter-individual variation in oral complex carbohydrate sensitivity (DTs and STs for both complex carbohydrates). Participants who were orally more sensitive or those who experienced high intensity for complex carbohydrates consumed more energy and starchy foods per day, and had bigger waist circumference measurements in comparison to participants who were orally less sensitive or experienced low intensity. Taken together, these results reveal a novel association between oral complex carbohydrate sensing and the consumption of complex carbohydrates. Whether or not oral sensitivity to complex carbohydrate influences waist circumference or vice versa still remains to be investigated.
Chapter Seven: Study 3(a) The Associations between Oral Complex Carbohydrate Sensitivity, BMI, Liking, and Consumption of Complex Carbohydrate Based Foods

7.1 Introduction

It is universally established that the human taste system consists of five basic tastes: sweet, sour, salty, bitter, umami, and much recent evidence supports a sixth taste responsive for fatty acids. (55-65) More recent psychophysics studies propose that humans may perceive complex carbohydrates, independent of sweet taste. (18, 21) That is, simple carbohydrates (glucose and sucrose) were not significantly correlated with complex carbohydrates, but significantly correlated with each other. (18, 21) Likewise, recent work from our laboratory (Chapter 5) demonstrated that humans are able to detect complex carbohydrates (maltodextrin, oligofructose) consistently in water, and individuals’ sensitivity towards these complex carbohydrates is qualitatively different from the five basic taste qualities (perceptual independence with sweet taste is at DT only, but overlap with sweetness at intensity).

Differences in the functionality of nutrient receptors within the mouth are known to impact liking (358-361) and intake of specific foods or ingredients (55, 253, 360, 362) associated with obesity has been a long-investigated area of study. A phenomenon, commonly described as ‘habituation’, has been observed in several studies investigating the consequences of exposure and consumption of specific nutrients on functionality of oral sensors to specific compounds. (255, 363) Such behaviour has been observed across several orally detected substances including, for
instance, sodium ion (e.g. sodium chloride); a negative relationship between oral sensitivity to sodium ion and habitual sodium intake has been recognised, that is, decreased sensitivity to sodium ion after repeated consumption of salty foods (e.g. Beauchamp et al. (364)). Other investigated nutrients include fatty acids (55, 60, 365) and simple carbohydrates, (116) but no one has previously investigated complex carbohydrates in this way. A recent cross-sectional study of 34 participants from our laboratory (Chapter 6) observed a positive correlation between oral sensitivity to complex carbohydrates, intake of complex carbohydrate-rich foods, as well as waist measurements. Although it is unclear why such findings were observed, it could be argued that the physiological regulation and functional significance of sensing low amounts of complex carbohydrate is beneficial to the survival of human beings, especially during times when foods are scarce, as complex carbohydrates represent a major source of energy for body functioning. Thus, it would be beneficial for humans to be able to sense complex carbohydrates in order to regulate glucose metabolism for functioning, in comparison to the functional significance of other nutrient detectors such as salt and fat where only small amounts are utilised by our cells. Nevertheless, as only one study (Chapter 6) has been conducted in this area, replication of this study using a larger sample size is important in order to be able to determine the generalisability of the previous results. Furthermore, in our previous chapters (Chapter 4 and Chapter 6), we did not include any measurements on the liking ratings of sweet and complex carbohydrate prototypical foods (both solutions versus prototypical foods). Thus, we were unable to rule out the possibility that liking may be influencing an individual’s consumption of sweet and complex carbohydrate based foods. Furthermore, it could be argued that liking of tastant solutions (e.g. sucrose
Chapter 7 – Study 3(a) The Associations between Oral Complex Carbohydrate Sensitivity, BMI, Liking, and Consumption of Complex Carbohydrate Based Foods

solutions) in a laboratory setting bears little relevance to actual intake of real food in everyday life. (318)

7.2 Aims, Hypotheses, and Terminologies

7.2.1 Aims

The aim of the study was to confirm whether humans could perceive complex carbohydrate (maltodextrin) from a range of concentration levels in a large sample group of adults. A secondary aim was to investigate associations between oral complex carbohydrate sensitivity, BMI, liking, and consumption of complex carbohydrate based foods; as well as investigate the associations between liking towards sweet and complex carbohydrate solutions and prototypical foods and consumption of sweet and complex carbohydrate based foods.

7.2.2 Hypotheses

- Participants will have detection threshold and suprathreshold intensity for complex carbohydrate (maltodextrin);
- There will be significant individual variance in oral complex carbohydrate sensitivity. Participants will be able to be classified into more sensitive/experienced high intensity, normal sensitive/experienced moderate intensity, and less sensitive/experienced low intensity groups according to the sensitivities of complex carbohydrate (maltodextrin);
- Oral suprathreshold intensity perception for complex carbohydrate will be positively associated with BMI, liking, and consumption of complex
Chapter 7 – Study 3(a) The Associations between Oral Complex Carbohydrate Sensitivity, BMI, Liking, and Consumption of Complex Carbohydrate Based Foods

carbohydrate based foods; but detection threshold for complex carbohydrate will not be associated with BMI and consumption of complex carbohydrate based foods;

- Liking towards complex carbohydrate prototypical foods will be positively associated with consumption of complex carbohydrate based foods; but liking towards complex carbohydrate solutions will not be associated with consumption of complex carbohydrate based foods;

- Liking towards sweet prototypical foods will be positively associated with consumption of sweet based foods; but liking towards sweet solutions will not be associated consumption of sweet based foods.

7.2.3 Terminologies

For consistency throughout this chapter, the terminology “oral complex carbohydrate sensitivity” refers to all types of complex carbohydrates and derivatives, while not diminishing the prospect that oral perception of complex carbohydrate could be due to textural differences. Although the terminology “polysaccharide taste” has been recommended by Sclafani (2) to denote to starch-derived saccharides containing three or more glucose unit, it can be confusing as the word “polysaccharide” is generally used to describe complex carbohydrate comprising more than ten monosaccharide units organised in chains. The word “oligosaccharide taste” (two to nine monosaccharide units) would be more appropriate terminology, nonetheless, it is not user friendly and it is unknown if perception of oligosaccharides is independent of texture differences. Therefore, at this stage of knowledge we recommend the use of “oral sensitivity to complex carbohydrate”, which correctly comprises all types of
complex carbohydrates and derivatives. Although dietary “carbohydrate” is an umbrella term for the monosaccharide and disaccharide sugars as well as starches and fibres, the term “sweet taste” has been collectively used to indicate sweetness. Thus “oral sensitivity to complex carbohydrate” would at the current state of knowledge be as correct as possible without oversimplifying tasting complex carbohydrates, but not easily confused with other sensations such as sweetness.

7.3 Subjects, Materials, and Methods

7.3.1 Subjects

Participants were recruited from a convenience sample of 138 students enrolled in a third-year Sensory Evaluation of Foods unit during 2016 at Deakin University, Melbourne campus, Australia. A total of 132 participants gave written informed consent and participated in the study (response rate = 96%). Data were excluded for individuals who: (1) were smokers ($n = 8$); (2) were pregnant or lactating ($n = 3$); (3) were taking any prescription medication that may interfere with their ability to taste ($n = 3$); or (4) had a history of any food allergy that may interfere with the study ($n = 11$) (Figure 7.1). Eight participants were also excluded from this study, as they did not attend all of the sessions. This study was approved by the institutional review board regulations of Deakin University (2012_162). The experimental protocol was also registered under the Australian New Zealand Clinical Trials Registry (ACTRN12617000551392), www.anzctr.org.au. This study also complies with the Declaration of Helsinki for Medical Research involving Human Subjects.
7.3.2 Study Design

Participants attended two laboratory sessions at Deakin University, separated by a wash-out period of at least seven days. Each session lasted about two hours, and participants were given breaks between tasks lasting from 15-30 minutes. The outlines of the two sessions are shown in Figure 7.2. During the sessions, detection threshold (DT) and suprathreshold intensity perception (ST) for glucose (sweet) and maltodextrin (complex carbohydrate), hedonic ratings for glucose and maltodextrin solutions, and hedonic ratings for a range of sweet and complex carbohydrate based foods were determined. Participants also participated in a general Labeled Magnitude...
Chapter 7 – Study 3(a) The Associations between Oral Complex Carbohydrate Sensitivity, BMI, Liking, and Consumption of Complex Carbohydrate Based Foods

Scale (gLMS) training session. Demographic information was also collected, including sex, age, and height and weight measurements. BMI (kg/m²) was calculated from the height and weight measurements. Participants also completed a Food Frequency Questionnaire (FFQ) and a Likes and Dislikes Questionnaire online within 1 week of sensory testing.

Psychophysics tasks (DT, ST), as well as hedonic ratings for a range of sweet and complex carbohydrate solutions were conducted in computerised, partitioned sensory booths in the Centre for Advanced Sensory Science using Compusense Cloud Software as part of the Compusense Academic Consortium (Compusense Inc., Ontario, Canada). Hedonic ratings for a range of sweet and complex carbohydrate based foods were conducted in individual workbenches at our teaching laboratory. Filtered deionised water was used as an oral rinsing agent. Participants were instructed to rinse their mouths with filtered deionised water for five seconds before beginning each task and between each sample set. To eliminate any visual or olfactory input, all testing sessions were conducted under red lighting, and participants were asked to wear nose clips during testing (except hedonic ratings). All solutions/foods were served at room temperature, with a three-digit code allocated to each sample. Participants were asked to abstain from eating and drinking (except room temperature water) for two hours prior to each session. Participants in the present study were part of a larger study focusing on the associations between sweet taste and oral complex carbohydrate sensitivity, liking, and consumption of *ad libitum* sweet and carbohydrate milkshakes (Chapter 8).
Figure 7.2 The study outline. The left chart represents the session outline for session one, the middle chart represents the session outline for session two, and the right chart represents the online questionnaires. Each session lasted about two hours. As the data collection was part of a laboratory class, participants were given intermittent breaks (teaching) in between each task lasting from 15-30 minutes.
7.3.3 Participant Training

Prior to using the general Labeled Magnitude Scale (gLMS) to rate taste intensity, participants were trained using the standard protocol outlined by Green et al. (266, 267) except the top of the scale was described as the strongest imaginable sensation of any kind (Appendix G, described in Chapter 2). (75). The 100-point scale comprised the following adjectives: ‘no sensation’ = 0, ‘barely detectable’ = 1.5, ‘weak’ = 6, ‘moderate’ =17, ‘strong’ = 35, ‘very strong’ = 52, and ‘strongest imaginable’ = 100. (75) Scales with only adjectives (not numbers) were presented to participants. During the training session, participants were taught to rate the intensity of the perceived sensation relative to a remembered or imagined sensation when using the gLMS scale. Participants were required to rate a list of seven remembered or imagined sensations, such as the warmth of the lukewarm water, the pain from biting of the tongue, and the sweetness of fairy floss (known as cotton candy in the USA, or candy floss in the UK).

7.3.4 Stimuli

Maltodextrin was used to investigate oral complex carbohydrate sensitivity (DTs and STs for complex carbohydrate; for details of stimuli see Table 7.1; detailed in Chapter 2). Maltodextrin with a dextrose equivalent (DE) of five was used in this study as it contains the lowest possible amount of free sugars (glucose, maltose) yet is soluble in water. DE is a measure of the percentage of reducing sugars relative to glucose on a dry basis. (269) Glucose was used to investigate sweet taste. All samples were prepared fresh on the day of testing using filtered deionised water (Cuno Filter
Chapter 7 – Study 3(a) The Associations between Oral Complex Carbohydrate Sensitivity, BMI, Liking, and Consumption of Complex Carbohydrate Based Foods Systems FS117S, Meriden, CT, USA) and stored in glass beakers at room temperature (20 ± 1 °C).

7.3.4.1 Analysis of Common Sugars in Maltodextrin Sample

To determine if the maltodextrin used in this study would be a suitable product, four percent w/v maltodextrin solutions were prepared for High Performance Liquid Chromatography (HPLC), as detailed in Chapter 2. The complex carbohydrate extracts were clarified with 25mL acetonitrile and filtered through a 0.45um filter into a 2mL vial. To determine the amount of common sugars in samples, filtered solutions were analysed by HPLC using amino column with an acetonitrile/water mobile phase containing salt and refractive index detection. Quantitation was made using a standard solution containing known amount of fructose, glucose, sucrose, maltose and lactose. Samples were measured in duplicate.

There were a total of 1.7g/100g (1.7% w/w) of common sugars for the maltodextrin used in this study (Glucose: 0.9% w/w) (Table 7.2). Detailed in Table 7.1 are the amount of glucose and total sugars (% w/v) present in each maltodextrin DT concentration. To verify that free sugars in complex carbohydrate solutions were below DT, if a participant was able to detect glucose in water (DT) at the lowest concentration (0.05 % w/v), potentially that would trigger detection for maltodextrin solution at step 6 (total sugars in maltodextrin: 0.032 % w/v) (Table 7.1).
Table 7.1 Sweetener and complex carbohydrate concentrations used for determination of detection thresholds.

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Concentration (% w/v)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td></td>
<td>0.05</td>
<td>0.09</td>
<td>0.1</td>
<td>0.2</td>
<td>0.4</td>
<td>0.6</td>
<td>1.1</td>
<td>1.8</td>
<td>2.9</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td></td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.6</td>
<td>1.1</td>
<td>1.9</td>
<td>3.6</td>
<td>6.3</td>
<td>11.2</td>
</tr>
<tr>
<td>Amount of Glucose in Maltodextrin ($10^{-3}$)</td>
<td></td>
<td>0.9</td>
<td>1.8</td>
<td>2.7</td>
<td>5.4</td>
<td>9.9</td>
<td>17.1</td>
<td>32.4</td>
<td>56.7</td>
<td>100.8</td>
</tr>
<tr>
<td>Amount of Total Sugars in Maltodextrin ($10^{-3}$)</td>
<td></td>
<td>1.7</td>
<td>3.4</td>
<td>5.1</td>
<td>10.2</td>
<td>18.7</td>
<td>32.3</td>
<td>61.2</td>
<td>107.1</td>
<td>190.4</td>
</tr>
</tbody>
</table>

The concentration series for glucose and maltodextrin were prepared with successive 0.25 log dilution steps. Reference chemical details: glucose (The Melbourne Food Depot, Melbourne, Australia); maltodextrin (Star-Dri 5, Tate & Lyle Ingredients Americas, USA). The ninth concentration was presented only when participants were unable to detect a difference from water solution in the previous eight. The amount of common and total sugars in maltodextrin concentrations were according to the report of analysis by the Australian Government National Measurement Institute from samples used in this study, where there were a total of 1.7g/100g (1.7% w/w) of free sugars for the maltodextrin (Glucose: 0.9% w/w).
Chapter 7 – Study 3(a) The Associations between Oral Complex Carbohydrate Sensitivity, BMI, Liking, and Consumption of Complex Carbohydrate Based Foods

Table 7.2 Common sugars composition of the maltodextrin used in the present study.

<table>
<thead>
<tr>
<th>Proximates</th>
<th>Maltodextrin (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0.9</td>
</tr>
<tr>
<td>Fructose</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>Sucrose</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>Maltose</td>
<td>0.8</td>
</tr>
<tr>
<td>Lactose</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>Total Sugars</td>
<td>1.7</td>
</tr>
</tbody>
</table>

These analyses were determined by the Australian Government National Measurement Institute, and were conducted by High Performance Liquid Chromatography (HPLC). 20g of each sample were sent for analyses.

7.3.5 Detection Threshold Determination for Sweet Taste and Oral Sensitivity to Complex Carbohydrates

DT was determined using the procedure outlined in the International Standards Organisation (ISO) Method of Investigating Sensitivity of Taste (described in Chapter 2). (265) Table 7.1 gives the nine concentrations used to assess DTs for sweet taste and oral complex carbohydrate sensitivity (the ninth concentration being presented only when participants were unable to detect a difference from water solution in the previous eight). (261) The concentration series for glucose and maltodextrin were prepared with successive 0.25 log dilution steps. (299) Concentration for maltodextrin was derived based on previous published findings of perceptually distinctive taste sensation concentration (i.e. see Lapis et al. (18) and systematic literature review by e Silva et al. (289)) without perceivable viscosity. After pilot testing, a concentration range between 0.11-11.2 percent (w/v) was used to measure DT levels for maltodextrin.

The eight samples for each stimulus were served in ascending concentration (15 mL per sample, in accordance with the standard ISO method), and each stimulus was presented to participants independently. Participants were unaware of the presentation order. Participants were instructed to taste each sample for five seconds...
then spit and rate whether: there was an absence of taste/oral perception (water-like); or if a taste/oral perception was identified but not recognised. (261) DT was defined as the concentration at which the participants selected the ‘taste/oral perception identified, but unknown taste quality/oral perception’. (261)

7.3.6 Suprathreshold Intensity Ratings for Glucose and Maltodextrin

Three concentrations (weak, medium, and strong) and a control (blank) solution were prepared to determine perceived suprathreshold intensity for glucose and maltodextrin, as described in Chapter 2 (Table 7.3). These concentrations were derived through informal bench-top testing (ascending intensity). The concentrations for each stimulus ranged from ‘weak’ to ‘strong’ on the gLMS. These samples were presented to participants in a randomised order.

Table 7.3 Concentrations (weak, medium, and strong intensity) of glucose and maltodextrin used for determination of suprathreshold intensity.

<table>
<thead>
<tr>
<th></th>
<th>Glucose</th>
<th>Maltodextrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak (% w/v)</td>
<td>5.3</td>
<td>3.6</td>
</tr>
<tr>
<td>Medium (% w/v)</td>
<td>10.6</td>
<td>6.3</td>
</tr>
<tr>
<td>Strong (% w/v)</td>
<td>21.2</td>
<td>11.2</td>
</tr>
<tr>
<td>Amount of Glucose in Maltodextrin (x10^3)</td>
<td>32.4</td>
<td>56.7</td>
</tr>
<tr>
<td>Amount of Total Sugars in Maltodextrin (x10^3)</td>
<td>61.2</td>
<td>107.1</td>
</tr>
</tbody>
</table>

Calculation of the amount of common and total sugars in maltodextrin concentrations were according to the report of analysis by the Australian Government National Measurement Institute from samples used in this study, where there were a total of 1.7g/100g (1.7% w/w) of free sugars for the maltodextrin (Glucose: 0.9% w/w).

7.3.7 Standardisation of gLMS Usage with Weight Ratings

To standardise gLMS usage within participants, a modified version of the method used by Delwiche et al. (268) was adapted for this study, as described in
Chapter 2. To control for idiosyncratic scale usage, participants were asked to rate the heaviness of six, visually identical weights (opaque bottles filled with sand and stone and completely wrapped in aluminium foil; weights of 53, 251, 499, 724, 897, and 1127g) in duplicates (Appendix I). Participants were asked to hold out their non-dominant hand palm up, while the experimenter placed the weighted bottle on the palm of the hand. Participants were instructed to rate the heaviness of each weight using the gLMS.

There was a significant correlation between the overall mean sweetness intensity ratings for glucose and overall mean heaviness ratings ($r = 0.38$, $P < 0.01$). Assuming that the intensity ratings of glucose and the heaviness of the bottles were unrelated, the significant correlation indicates that the gLMS ratings were subject to differences in individual scale-use and thus require standardisation across participants. (54, 70, 268) To determine a personal standardisation factor, the grand mean for heaviness across weight levels and participants was divided by each participant’s average intensity for heaviness. (54) Each individual’s sweet (glucose) taste intensity and sweetness intensity ratings were multiplied by his or her personal standardisation factor for scale-use bias. (54, 268)
Chapter 7 – Study 3(a) The Associations between Oral Complex Carbohydrate Sensitivity, BMI, Liking, and Consumption of Complex Carbohydrate Based Foods

7.3.8 Hedonic Ratings for Sweet and Complex Carbohydrate Solutions and Prototypical Foods

To measure liking of glucose and maltodextrin solutions, three concentrations (weak, medium, and strong) and a control (blank) solution were prepared and presented to participants in a randomised order, as discussed in Chapter 2 (Table 7.3). These solutions were identical to the concentrations used to assess suprathreshold intensity ratings for glucose and maltodextrin.

To assess liking of sweet and complex carbohydrate prototypical foods, participants were required to rate liking of 16 food items (eight sweet taste and eight complex carbohydrate based foods). The foods included in testing had approximately equivalent fat per 100g. Participants were given a variety of different sweet and complex carbohydrate based foods representing a range of dietary carbohydrate contents per serve (differences in grams of sugar or starch per 100g), approximately equivalent to the concentrations (% w/v) used to measure suprathreshold intensity ratings for glucose and maltodextrin. Eight small samples (5-20g) per tray were served in a randomised order, and each tray was presented to participants independently. The foods included in testing can be viewed in Table 7.4 (Appendix L).

Liking of both solutions and foods was measured using a nine-point hedonic scale ranging from 1 = dislike extremely to 9 = like extremely (Appendix M). All liking evaluations were conducted without the use of nose clips and following psychophysics tests. All solutions/foods were ingested.
### Table 7.4 Sweet and complex carbohydrate based foods used for hedonic ratings.

<table>
<thead>
<tr>
<th>Food</th>
<th>Sugar per 100g</th>
<th>Starch per 100g</th>
<th>Fat per 100g</th>
<th>Amount provided (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red kidney beans, unsalted, canned</td>
<td>0.7</td>
<td>13.1</td>
<td>0.5</td>
<td>20g</td>
</tr>
<tr>
<td>Pasta, elbow, boiled, plain</td>
<td>0</td>
<td>28.4</td>
<td>0.3</td>
<td>20g</td>
</tr>
<tr>
<td>Rice, white, boiled with water</td>
<td>0.1</td>
<td>36.0</td>
<td>0</td>
<td>20g</td>
</tr>
<tr>
<td>White bread</td>
<td>3.5</td>
<td>48.0</td>
<td>1.8</td>
<td>20g</td>
</tr>
<tr>
<td>Weet-Bix (Australian breakfast cereal biscuit)</td>
<td>3.3</td>
<td>67.0</td>
<td>1.4</td>
<td>20g</td>
</tr>
<tr>
<td>Pretzel chips, low fat</td>
<td>1.9</td>
<td>72.8</td>
<td>1.6</td>
<td>20g</td>
</tr>
<tr>
<td>Rice cake, thin, plain</td>
<td>0.2</td>
<td>78.0</td>
<td>2.8</td>
<td>20g</td>
</tr>
<tr>
<td>Rice cracker, plain</td>
<td>4.7</td>
<td>81.5</td>
<td>1.1</td>
<td>20g</td>
</tr>
<tr>
<td>Tomato puree (passata), unsalted, canned</td>
<td>8.2</td>
<td>0</td>
<td>1.1</td>
<td>15mL</td>
</tr>
<tr>
<td>Apples, dried</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>20g</td>
</tr>
<tr>
<td>Gelatin dessert, blackcurrant flavoured</td>
<td>19.5</td>
<td>0</td>
<td>0</td>
<td>15g</td>
</tr>
<tr>
<td>Chocolate flavoured syrup, low fat</td>
<td>54.8</td>
<td>0</td>
<td>0.4</td>
<td>15mL</td>
</tr>
<tr>
<td>Strawberry jam</td>
<td>64.0</td>
<td>0</td>
<td>0</td>
<td>15mL</td>
</tr>
<tr>
<td>Raisins</td>
<td>71.4</td>
<td>0</td>
<td>0</td>
<td>20g</td>
</tr>
<tr>
<td>Honey</td>
<td>82.5</td>
<td>0</td>
<td>0</td>
<td>15mL</td>
</tr>
<tr>
<td>Fairy floss (known as cotton candy in the USA, or candy floss in the UK)</td>
<td>97.2</td>
<td>0</td>
<td>0</td>
<td>5g</td>
</tr>
</tbody>
</table>

Food details: Red kidney beans, unsalted, canned (Coles Homebrand, Coles, Australia); Pasta, elbow (Coles Homebrand, Coles, Australia); Rice, white (SunRice, Ricegrowers Limited, Australia); White bread (Coles Homebrand, Coles, Australia); Weet-Bix (Sanitarium Health and Wellbeing Company, Australia); Pretzel chips, low fat (Parker’s, The Smith’s Snackfood Company, Australia); Rice cake, thin, plain (SunRice, Ricegrowers Limited, Australia); Rice cracker, plain (Sakata, The Smith’s Snackfood Company, Australia); Tomato puree (passata), unsalted, canned (Coles Homebrand, Coles, Australia); Apples, dried (Angas Park, Angas Park Fruit Co, Australia); Gelatin dessert, blackcurrant flavoured (Aeroplane Jelly, McCormick & Company, Australia); chocolate flavoured syrup, low fat (Cottee’s, Heinz Foodservice, Australia); Strawberry jam (IXL, SPC Ardmona, Australia); Raisins (Coles Homebrand, Coles, Australia); Honey (Coles Homebrand, Coles, Australia); Fairy floss (The Fairy Floss King, NSW, Australia).
7.3.9 Standardisation of Hedonic Scale Usage with Non-Food Items

To control for idiosyncratic scale usage, participants were asked to complete a Likes and Dislikes Questionnaire (Appendix N), as described in Chapter 2. Participants were required to rate, on average, how much they liked or disliked a number of food/beverage items and common experiences across ten categories (77 items; i.e. grains/cereals, meat/meat alternatives, fast foods, dairy, fruit and vegetables, snack foods, fats/oils, beverages, oral sensation, non-food) on a nine-point hedonic scale. Examples of non-food items (4 items) included how much they liked or disliked jumping in a pool on a hot day, or the glare of headlights.

There was a significant correlation between the overall mean hedonic ratings for food/beverage items and overall mean hedonic ratings for non-food items ($r = 0.22, P < 0.05$). As individual hedonic ratings for food/beverage items and non-food items were assumed to be unrelated, the significant correlation indicated that the hedonic scale ratings were subject to differences in individual scale-use and required standardisation across participants. To determine a personal standardisation factor, a similar calculation method with gLMS standardisation was used (i.e. the grand mean for non-food items across all participants was divided by each participant’s average hedonic ratings for non-food items). Each individual’s hedonic ratings were multiplied by his or her personal standardisation factor for scale-use bias.

7.3.10 Body Composition

All participants were asked to remove shoes and heavy clothing to ensure accurate measurements, as discussed in Chapter 2. All body composition measurements were measured first thing during the first session after a 2-hour fast
Chapter 7 – Study 3(a) The Associations between Oral Complex Carbohydrate Sensitivity, BMI, Liking, and Consumption of Complex Carbohydrate Based Foods (food only). Participants’ body weight was self-measured to the nearest 0.1 kg using a segmental body composition analyser (TBF-300A) (Tanita Corporation, Tokyo, Japan). Participants’ height was self-measured to the nearest 0.1 cm using a portable stadiometer (Seca213) (Seca, Hamburg, Germany). All measurements were repeated twice to ensure accuracy. Average height and weight measurements were used to calculate BMI (weight in kg/m²) and determine weight status (i.e. normal weight or overweight/obese). Weight statuses were defined under World Health Organisation BMI classification. (277)

7.3.11 Dietary Intake

An adapted version of the 1995 Australian National Nutrition Survey FFQ (284) was used to measure each participant’s habitual pattern of food intake, as described in Chapter 2 (Appendix P). Participants were required to indicate, on average, how many times in the previous month they consumed a number of food and beverages and vitamin and mineral supplements (118 items; bread and cereal foods, dairy foods, meat, fish, eggs, sweets, baked goods, and snacks, dressings, non-dairy beverages, vegetables, fruits). Participants were instructed to select the most appropriate answer on a nine-point scale with response options ‘1 = never or less than once per month’, ‘2 = 1-3 times per month’, ‘3 = once per week’, ‘4 = 2-4 times per week’, ‘5 = 5-6 times per week’, ‘6 = once per day’, ‘7 = 2-3 times per day’, ‘8 = 4-5 times per day’, and ‘9 = 6 or more times per day’.
Chapter 7 – Study 3(a) The Associations between Oral Complex Carbohydrate Sensitivity, BMI, Liking, and Consumption of Complex Carbohydrate Based Foods

7.3.12 Statistical analysis

Statistical analysis was performed using IBM SPSS statistical software version 23.0 (SPSS, Chicago, IL, USA). Data are presented as means with standard errors (SEM). For STs and hedonic ratings for solutions, the geometric mean score of the three ratings (weak, medium, and strong) was calculated. For hedonic ratings of a range of sweet and complex carbohydrate prototypical foods, a geometric mean score of the eight food items was calculated. Spearman’s rank correlation coefficient was calculated between DTs and STs with a criterion for statistical significance set at $P < 0.05$ for psychophysics analyses.

Descriptive statistics were employed to describe demographic information, sweetener (glucose) and complex carbohydrate (maltodextrin) DTs and STs, hedonic ratings for sweet and complex carbohydrate solutions and prototypical foods, and BMI. DTs for glucose and maltodextrin were treated as grouping variables (tertiles) with participants categorised as more sensitive (1/3), normal sensitive (2/3), and less sensitive (3/3) to explore differences between continuous (BMI, liking) and categorical (frequency consumption of sweet and carbohydrate based foods) variables. STs for glucose and maltodextrin were treated as grouping variables (tertiles) with participants categorised as those who experienced low intensity (1/3), moderate intensity (2/3), and high intensity (3/3) to explore differences between continuous (BMI, liking) and categorical (frequency consumption of sweet and carbohydrate based foods) variables. DTs and STs for glucose and maltodextrin were grouped into tertiles to allow comparison of most and least sensitive groupings or those groups who experienced low and high intensity (*i.e.* four sets of tertiles were determined: one for DT for glucose and maltodextrin, and one for ST for glucose and maltodextrin). Similarly, individuals’ hedonic ratings for sweet and complex carbohydrate solutions and
prototypical foods were treated as grouping variables (tertiles) with participants categorised as those who rated low (1/3), moderate (2/3) and high (3/3) on the hedonic scale to explore differences between variables (BMI, frequency consumption of sweet and carbohydrate based foods). Hedonic ratings for sweet and complex carbohydrate solutions and prototypical foods were grouped into tertiles to allow comparison of those groups who rated low and high on the hedonic scale (i.e. four sets of tertiles were determined for hedonic ratings: for sweet solutions, sweet prototypical foods, complex carbohydrate solutions, and complex carbohydrate prototypical foods). (299) An independent t-test was used to detect differences in BMI between more sensitive and less sensitive participants or those who experienced low and high intensity (low and high tertile groups). (299) Chi-square test for independence was used to detect differences in frequency of consumption of sweet or complex carbohydrate based foods between DTs and STs for glucose and maltodextrin groups. An independent t-test was used to detect differences in BMI between those who rated low and high on the hedonic scale groups (low and high tertile groups). Chi-square test for independence was used to detect differences in frequency of consumption of sweet or complex carbohydrate based foods between hedonic groups. Pearson’s product-moment correlations were also conducted to analyse the relationships between sweet taste function and oral complex carbohydrate sensitivity (DTs and STs for glucose and maltodextrin), hedonic ratings for sweet and complex carbohydrate solutions and prototypical foods, and BMI. Independent t-tests were used to analyse differences in terms of sweet taste function and oral complex carbohydrate sensitivity (DTs and STs for glucose and maltodextrin), liking, and BMI between gender groups. Significance was accepted at $P \leq 0.01$ to reduce the possibility of making a type I error due to multiple tests being conducted. (299) The p-value was not adjusted for multiple
Chapter 7 – Study 3(a) The Associations between Oral Complex Carbohydrate Sensitivity, BMI, Liking, and Consumption of Complex Carbohydrate Based Foods

comparisons by the application of Bonferroni or other equivalent method, as these approaches can be overly conservative (increasing risk of type II error) and can potentially mask important findings. (315, 316) In order to conduct the appropriate statistical analyses, response options for consumption variables were collapsed. (285) For example, the white bread category was recoded from the original nine response options, down to three response options (Appendix Q).

7.4 Results

7.4.1 Participants

Baseline characteristics of the 99 participants who completed the study are detailed in Table 7.5. There were no significant differences in BMI and age between male and female participants (all P > 0.05).

7.4.2 Sweet Taste Function and Oral Complex Carbohydrate Sensitivity

There were no significant differences in sweet taste function and oral complex carbohydrate (DTs and STs for glucose and maltodextrin) between male and female participants (all P > 0.05); therefore, the data are presented together. The mean DTs and STs, standard errors, and range for both glucose and maltodextrin are presented in Table 7.6.
Table 7.5 Baseline characteristics of study participants (Mean values and standard errors).

<table>
<thead>
<tr>
<th></th>
<th>All (n = 99)</th>
<th>Normal Weight (n = 82)</th>
<th>Overweight/Obese (n = 17)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>Age (years)</td>
<td>23.6 ± 0.5</td>
<td>23.5 ± 0.5</td>
<td>24.2 ± 1.8</td>
</tr>
<tr>
<td>Gender (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>92 ± 77</td>
<td>77 ± 15</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>Male</td>
<td>7 ± 5</td>
<td>5 ± 2</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165.9 ± 0.8</td>
<td>165.4 ± 0.9</td>
<td>168.2 ± 2.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>62.1 ± 1.2</td>
<td>58.5 ± 0.9</td>
<td>79.5 ± 3.4</td>
</tr>
<tr>
<td>BMI (kg/m^2) (^1)</td>
<td>22.6 ± 0.3</td>
<td>21.5 ± 0.2</td>
<td>28.0 ± 0.8</td>
</tr>
<tr>
<td>BMI range (kg/m^2) (^1)</td>
<td>18.5-37.9</td>
<td>18.5-24.9</td>
<td>25.1-37.9</td>
</tr>
</tbody>
</table>

\(^1\)Normal weight, BMI = 18.5-24.9 kg/m^2; overweight, BMI = 25-29.9 kg/m^2; obese, BMI ≥ 30 kg/m^2. (277)

Table 7.6 Detection thresholds (% w/v) and mean intensity ratings (gLMS) for glucose and maltodextrin presented as mean, standard error of mean, and range.

<table>
<thead>
<tr>
<th></th>
<th>Detection Threshold (n = 99)</th>
<th>Mean Intensity Rating (n = 99)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>Range</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.8 ± 0.07</td>
<td>0.05 - 1.8</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>3.2 ± 0.30</td>
<td>0.11 - 11.2</td>
</tr>
</tbody>
</table>
Chapter 7 – Study 3(a) The Associations between Oral Complex Carbohydrate Sensitivity, BMI, Liking, and Consumption of Complex Carbohydrate Based Foods

There was large individual variation among the participants (Figure 7.3). For example DT for maltodextrin ranged from 0.11 % w/v to 11.2 % w/v. Figure 7.4 shows the psychophysical functions of the group mean and examples of an individual who experienced higher intensity and an individual who experienced lower intensity when tasting glucose and maltodextrin.

Figure 7.3 Frequency distribution of detection thresholds for: a) glucose, b) maltodextrin.
Figure 7.4 Psychophysical curves of the group mean and examples of a participant experiencing high intensity and a participant experiencing low intensity for (a) Glucose (b) Maltodextrin. Included in each graph is the mean psychophysical curve, as well as an example of a participant experiencing higher intensity (highest curve) and a participant experiencing lower intensity (lowest curve) for glucose and maltodextrin. The y-axis is a numerical measure of intensity from the gLMS. The x-axis is the actual concentration in % w/v.
7.4.3 Relationships between Oral Complex Carbohydrate Sensitivity and Sweet Taste Function

The DTs of glucose and maltodextrin were weakly correlated with one another ($r = 0.33$, $P < 0.001$; Figure 7.5a). For each participant, there were moderate correlations between the mean intensity ratings of glucose and maltodextrin ($r = 0.42$, $P < 0.001$) (Figure 7.5b).

Participants were stratified into tertile groups according to the stimuli (glucose, maltodextrin) tested and all taste measures (DT, ST). Across the same measures, those who were able to detect maltodextrin in water at low concentrations (lower tertile; $n = 18$) were also more sensitive to glucose. Interestingly, five participants were able to detect maltodextrin in water at lower concentrations, but were less sensitive to glucose. Looking at the concentrations, it is likely that they detected the complex carbohydrates in the sample rather than any free sugars in the complex carbohydrate sample. For example, one participant was able to detect maltodextrin at 0.2% w/v (Glucose: 0.0018% w/v, total sugars in maltodextrin: 0.0034% w/v) but only able to detect glucose at 1.8% w/v. For intensity ratings, some participants experienced higher intensity (higher tertile; $n = 15$) or lower intensity ($n = 17$) for both glucose and maltodextrin. Seven participants experienced higher intensity for maltodextrin, but experienced lower intensity to glucose. Of particular note, no participant was more sensitive or less sensitive to both glucose and maltodextrin across all taste measures.
Figure 7.5  (a) Spearman rank correlations between detection thresholds of glucose and maltodextrin.  (b) Spearman rank correlations between intensity ratings of glucose and maltodextrin. The solid line in each graph represents the regression line. **$P \leq 0.01$. 

(a)

(b)
7.4.4 Hedonic Ratings for Sweet and Complex Carbohydrate Solutions and Prototypical Foods

Figure 7.6 shows the mean liking ratings for both sweet and complex carbohydrate solutions and prototypical foods.

7.4.5 Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, and Hedonic Ratings for Sweet and Complex Carbohydrate Solutions and Prototypical Foods

No significant correlations were identified between any measures of sweet taste function (DT and ST), oral complex carbohydrate sensitivity (DT and ST), with hedonic ratings for sweet and complex carbohydrate solutions and prototypical foods (all \( P > 0.05 \)). Similarly, when grouped into tertiles according to their sweet taste function and oral complex carbohydrate sensitivity, there were no significant differences in hedonic ratings for sweet and complex carbohydrate solutions and prototypical foods between the more sensitive and less sensitive participants or participants who experienced high intensity and low intensity for either glucose or maltodextrin (all \( P > 0.05 \)).
Figure 7.6 Mean hedonic ratings for a range of (a) sweet solutions, (b) complex carbohydrate solutions, (c) sweet prototypical foods, and (d) complex carbohydrate prototypical foods. The y-axis is the adjusted liking ratings from a nine-point hedonic scale. The x-axis represents the different sweet and complex carbohydrate concentrations and food commodities measured.
Chapter 7 – Study 3(a) The Associations between Oral Complex Carbohydrate Sensitivity, BMI, Liking, and Consumption of Complex Carbohydrate Based Foods

(a)

(b)

(c)

(d)

Sweet (glucose) solutions

Complex carbohydrate (maltodextrin) solutions

Sweet prototypical foods

Complex carbohydrate prototypical foods
7.4.6 Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, and BMI

No significant correlations were identified between any measures of sweet taste function (DT and ST), oral complex carbohydrate sensitivity (DT and ST), with BMI for both glucose and maltodextrin (all $P \geq 0.01$). Similarly, when grouped into tertiles according to their sweet taste function and oral complex carbohydrate sensitivity, there were no significant differences in BMI between the more sensitive and less sensitive participants or participants who experienced high intensity or low intensity for either glucose and maltodextrin (all $P \geq 0.01$) (Figure 7.7a and Figure 7.7b). When stratified into weight status, there were no significant differences in any measure of sweet taste function (DT and ST for glucose) and oral complex carbohydrate sensitivity (DT and ST for maltodextrin) between normal weight and overweight/obese participants (all $P \geq 0.01$).

7.4.7 Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, and Frequency of Consumption of Sweet and Complex Carbohydrate Based Foods

There were no significant differences between measures of sweet taste function (DT and ST) and the frequency of consumption of sweet foods measured (all $P \geq 0.01$; Table 7.7). Similarly, there were no significant differences between measures of oral complex carbohydrate sensitivity (DT and ST) and the frequency of consumption of complex carbohydrate foods measured (all $P \geq 0.01$; Table 7.8).
Chapter 7 – Study 3(a) The Associations between Oral Complex Carbohydrate Sensitivity, BMI, Liking, and Consumption of Complex Carbohydrate Based Foods

**Figure 7.7 (A-B)** BMI mean and standard errors between more sensitive and less sensitive participants and those who experienced high intensity and low intensity according to sweet taste function and oral complex carbohydrate sensitivity measures. **(C-D)** BMI mean and standard errors between participants with high hedonic ratings and low hedonic ratings for both sweet and complex carbohydrate solutions and prototypical foods.
Table 7.7 *Sweet taste function (tertile groups) and frequency of reported sweet foods consumption.*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Detection threshold</th>
<th>Suprathreshold intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confectionaries, other than chocolate (serves consumed within an average month)</td>
<td>$\chi^2(2) = 0.69, P = 0.70$</td>
<td>$\chi^2(2) = 0.96, P = 0.61$</td>
</tr>
<tr>
<td>Jam, marmalade, syrup, honey</td>
<td>$\chi^2(2) = 0.50, P = 0.78$</td>
<td>$\chi^2(2) = 2.63, P = 0.27$</td>
</tr>
<tr>
<td>Fruit juice</td>
<td>$\chi^2(2) = 4.99, P = 0.08$</td>
<td>$\chi^2(2) = 9.79, P = 0.02$</td>
</tr>
<tr>
<td>Vegetable, tomato juice</td>
<td>$\chi^2(2) = 5.64, P = 0.06$</td>
<td>$\chi^2(2) = 1.67, P = 0.43$</td>
</tr>
<tr>
<td>Fruit juice drink or fruit drink</td>
<td>$\chi^2(2) = 3.08, P = 0.22$</td>
<td>$\chi^2(2) = 2.14, P = 0.34$</td>
</tr>
<tr>
<td>Low-joule cordial</td>
<td>$\chi^2(2) = 3.86, P = 0.15$</td>
<td>$\chi^2(2) = 1.14, P = 0.57$</td>
</tr>
<tr>
<td>Cordial</td>
<td>$\chi^2(2) = 3.85, P = 0.15$</td>
<td>$\chi^2(2) = 0.55, P = 0.76$</td>
</tr>
<tr>
<td>Low-Joule soft drink</td>
<td>$\chi^2(2) = 0.51, P = 0.78$</td>
<td>$\chi^2(2) = 2.33, P = 0.31$</td>
</tr>
<tr>
<td>Soft drinks (including flavoured mineral water)</td>
<td>$\chi^2(2) = 0.22, P = 0.90$</td>
<td>$\chi^2(2) = 0.93, P = 0.63$</td>
</tr>
<tr>
<td>Apple or pear</td>
<td>$\chi^2(4) = 3.76, P = 0.44$</td>
<td>$\chi^2(4) = 1.13, P = 0.89$</td>
</tr>
<tr>
<td>Orange, mandarin or grapefruit</td>
<td>$\chi^2(4) = 6.27, P = 0.18$</td>
<td>$\chi^2(4) = 4.48, P = 0.35$</td>
</tr>
<tr>
<td>Banana</td>
<td>$\chi^2(4) = 1.64, P = 0.80$</td>
<td>$\chi^2(4) = 3.77, P = 0.44$</td>
</tr>
<tr>
<td>Peach, nectarine, plum, or apricot</td>
<td>$\chi^2(4) = 1.90, P = 0.75$</td>
<td>$\chi^2(4) = 2.33, P = 0.66$</td>
</tr>
<tr>
<td>Grapes or berries</td>
<td>$\chi^2(4) = 6.35, P = 0.17$</td>
<td>$\chi^2(4) = 2.16, P = 0.71$</td>
</tr>
<tr>
<td>Melon (<em>e.g.</em> watermelon, rockmelon, honeydew melon)</td>
<td>$\chi^2(4) = 10.76, P = 0.02$</td>
<td>$\chi^2(4) = 4.95, P = 0.29$</td>
</tr>
<tr>
<td>Pineapple</td>
<td>$\chi^2(2) = 2.27, P = 0.70$</td>
<td>$\chi^2(2) = 2.65, P = 0.27$</td>
</tr>
<tr>
<td>Mango</td>
<td>$\chi^2(2) = 0.11, P = 0.95$</td>
<td>$\chi^2(2) = 6.80, P = 0.03$</td>
</tr>
<tr>
<td>Chocolate</td>
<td>$\chi^2(4) = 6.55, P = 0.16$</td>
<td>$\chi^2(4) = 3.26, P = 0.52$</td>
</tr>
<tr>
<td>Cakes, sweet muffins, scones or pikelets</td>
<td>$\chi^2(4) = 6.55, P = 0.16$</td>
<td>$\chi^2(4) = 2.09, P = 0.70$</td>
</tr>
<tr>
<td>Sweet pies or sweet pastries</td>
<td>$\chi^2(4) = 6.67, P = 0.16$</td>
<td>$\chi^2(4) = 3.80, P = 0.44$</td>
</tr>
<tr>
<td>Other puddings or desserts</td>
<td>$\chi^2(4) = 5.13, P = 0.27$</td>
<td>$\chi^2(4) = 2.51, P = 0.64$</td>
</tr>
<tr>
<td>Plain sweet biscuits</td>
<td>$\chi^2(4) = 7.67, P = 0.11$</td>
<td>$\chi^2(4) = 2.93, P = 0.57$</td>
</tr>
<tr>
<td>Cream chocolate biscuits</td>
<td>$\chi^2(4) = 1.72, P = 0.77$</td>
<td>$\chi^2(4) = 3.78, P = 0.44$</td>
</tr>
</tbody>
</table>
Table 7.8 *Oral complex carbohydrate sensitivity (tertile groups) and frequency of reported complex carbohydrate based foods consumption.*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Detection threshold</th>
<th>Suprathreshold intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>White bread, toast, or rolls (serves consumed within an average month)</td>
<td>$\chi^2(4) = 3.18, P = 0.53$</td>
<td>$\chi^2(4) = 2.76, P = 0.59$</td>
</tr>
<tr>
<td>Wholemeal/mixed grain bread, toast or roll</td>
<td>$\chi^2(4) = 5.61, P = 0.23$</td>
<td>$\chi^2(4) = 3.19, P = 0.53$</td>
</tr>
<tr>
<td>Muesli</td>
<td>$\chi^2(4) = 2.36, P = 0.67$</td>
<td>$\chi^2(4) = 2.52, P = 0.64$</td>
</tr>
<tr>
<td>Breakfast cereal</td>
<td>$\chi^2(4) = 3.74, P = 0.44$</td>
<td>$\chi^2(4) = 3.77, P = 0.44$</td>
</tr>
<tr>
<td>Rice (including white or brown)</td>
<td>$\chi^2(4) = 12.27, P = 0.02$</td>
<td>$\chi^2(4) = 6.36, P = 0.17$</td>
</tr>
<tr>
<td>English muffin, bagel, or crumpet</td>
<td>$\chi^2(2) = 0.02, P = 0.99$</td>
<td>$\chi^2(2) = 3.77, P = 0.15$</td>
</tr>
<tr>
<td>Dry or savoury biscuits</td>
<td>$\chi^2(2) = 0.68, P = 0.71$</td>
<td>$\chi^2(2) = 0.41, P = 0.81$</td>
</tr>
<tr>
<td>Crispbread crackers</td>
<td>$\chi^2(2) = 0.51, P = 0.78$</td>
<td>$\chi^2(2) = 0.51, P = 0.78$</td>
</tr>
<tr>
<td>Cooked porridge</td>
<td>$\chi^2(2) = 0.14, P = 0.93$</td>
<td>$\chi^2(2) = 1.62, P = 0.45$</td>
</tr>
<tr>
<td>Pasta (including filled), and noodles</td>
<td>$\chi^2(2) = 1.63, P = 0.44$</td>
<td>$\chi^2(2) = 0.70, P = 0.70$</td>
</tr>
<tr>
<td>Potato, boiled, mashed, baked</td>
<td>$\chi^2(2) = 1.91, P = 0.39$</td>
<td>$\chi^2(2) = 4.79, P = 0.09$</td>
</tr>
<tr>
<td>Pumpkin</td>
<td>$\chi^2(2) = 0.38, P = 0.83$</td>
<td>$\chi^2(2) = 6.02, P = 0.04$</td>
</tr>
<tr>
<td>Sweet potato</td>
<td>$\chi^2(2) = 0.39, P = 0.82$</td>
<td>$\chi^2(2) = 2.27, P = 0.32$</td>
</tr>
<tr>
<td>Peas</td>
<td>$\chi^2(2) = 6.35, P = 0.04$</td>
<td>$\chi^2(2) = 0.46, P = 0.79$</td>
</tr>
<tr>
<td>Other beans, lentils</td>
<td>$\chi^2(2) = 0.39, P = 0.82$</td>
<td>$\chi^2(2) = 0.15, P = 0.93$</td>
</tr>
<tr>
<td>Hot chips</td>
<td>$\chi^2(4) = 5.83, P = 0.21$</td>
<td>$\chi^2(4) = 1.41, P = 0.84$</td>
</tr>
<tr>
<td>Potato chips, corn chips, Twisties, etc.</td>
<td>$\chi^2(2) = 0.45, P = 0.98$</td>
<td>$\chi^2(2) = 2.73, P = 0.60$</td>
</tr>
<tr>
<td>Cakes, sweet muffins, scones or pikelets</td>
<td>$\chi^2(4) = 3.97, P = 0.41$</td>
<td>$\chi^2(4) = 1.69, P = 0.79$</td>
</tr>
<tr>
<td>Sweet pies or sweet pastries</td>
<td>$\chi^2(4) = 3.79, P = 0.44$</td>
<td>$\chi^2(4) = 0.76, P = 0.94$</td>
</tr>
<tr>
<td>Other puddings or desserts</td>
<td>$\chi^2(4) = 0.38, P = 0.98$</td>
<td>$\chi^2(4) = 0.76, P = 0.95$</td>
</tr>
<tr>
<td>Plain sweet biscuits</td>
<td>$\chi^2(4) = 0.68, P = 0.95$</td>
<td>$\chi^2(4) = 0.69, P = 0.14$</td>
</tr>
<tr>
<td>Cream chocolate biscuits</td>
<td>$\chi^2(4) = 3.75, P = 0.44$</td>
<td>$\chi^2(4) = 5.07, P = 0.28$</td>
</tr>
</tbody>
</table>
7.4.8 Liking of Sweet and Complex Carbohydrate Solutions and Prototypical Foods, BMI, and Frequency of Consumption of Sweet and Carbohydrate Based Foods

No significant correlations were identified between liking for sweet and complex carbohydrate solutions and prototypical foods with BMI (all $P \geq 0.01$). Similarly, when grouped into tertiles (according to liking ratings towards solutions and prototypical foods), there were no significant differences in BMI between groups (all $P \geq 0.01$) (Figure 7.7c and Figure 7.7d). When stratified into BMI categories, there were no significant differences in any measure of liking (solutions and prototypical foods) between normal weight and overweight/obese participants (all $P \geq 0.01$).

There were no significant differences between measures of sweet and complex carbohydrate hedonic ratings (solutions, prototypical foods) and the frequency of consumption of any of the sweet and complex carbohydrate based foods measured (all $P \geq 0.01$; Table 7.9 and Table 7.10).
Table 7.9 Hedonic ratings (tertile groups) and frequency of reported sweet foods consumption.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hedonic Ratings of Sweet Solutions</th>
<th>Hedonic Ratings of Sweet Foods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confectionaries, other than chocolate (serves consumed within an average month)</td>
<td>$\chi^2 (2) = 0.54, P = 0.76$</td>
<td>$\chi^2 (2) = 1.59, P = 0.45$</td>
</tr>
<tr>
<td>Jam, marmalade, syrup, honey</td>
<td>$\chi^2 (2) = 2.10, P = 0.35$</td>
<td>$\chi^2 (2) = 1.19, P = 0.55$</td>
</tr>
<tr>
<td>Fruit juice</td>
<td>$\chi^2 (2) = 8.48, P = 0.02$</td>
<td>$\chi^2 (2) = 1.62, P = 0.45$</td>
</tr>
<tr>
<td>Vegetable, tomato juice</td>
<td>$\chi^2 (2) = 1.62, P = 0.45$</td>
<td>$\chi^2 (2) = 0.86, P = 0.65$</td>
</tr>
<tr>
<td>Fruit juice drink or fruit drink</td>
<td>$\chi^2 (2) = 5.24, P = 0.07$</td>
<td>$\chi^2 (2) = 2.14, P = 0.34$</td>
</tr>
<tr>
<td>Low-joule cordial</td>
<td>$\chi^2 (2) = 2.10, P = 0.35$</td>
<td>$\chi^2 (2) = 1.19, P = 0.45$</td>
</tr>
<tr>
<td>Cordial</td>
<td>$\chi^2 (2) = 3.85, P = 0.15$</td>
<td>$\chi^2 (2) = 0.47, P = 0.79$</td>
</tr>
<tr>
<td>Low-Joule soft drink</td>
<td>$\chi^2 (2) = 0.99, P = 0.60$</td>
<td>$\chi^2 (2) = 1.82, P = 0.40$</td>
</tr>
<tr>
<td>Soft drinks (including flavoured mineral water)</td>
<td>$\chi^2 (2) = 1.94, P = 0.38$</td>
<td>$\chi^2 (2) = 0.93, P = 0.63$</td>
</tr>
<tr>
<td>Apple or pear</td>
<td>$\chi^2 (4) = 4.46, P = 0.35$</td>
<td>$\chi^2 (4) = 4.43, P = 0.36$</td>
</tr>
<tr>
<td>Orange, mandarin or grapefruit</td>
<td>$\chi^2 (4) = 2.06, P = 0.72$</td>
<td>$\chi^2 (4) = 2.99, P = 0.56$</td>
</tr>
<tr>
<td>Banana</td>
<td>$\chi^2 (4) = 8.25, P = 0.08$</td>
<td>$\chi^2 (4) = 6.69, P = 0.15$</td>
</tr>
<tr>
<td>Peach, nectarine, plum, or apricot</td>
<td>$\chi^2 (4) = 4.41, P = 0.35$</td>
<td>$\chi^2 (4) = 0.91, P = 0.93$</td>
</tr>
<tr>
<td>Grapes or berries</td>
<td>$\chi^2 (4) = 6.15, P = 0.19$</td>
<td>$\chi^2 (4) = 1.089, P = 0.89$</td>
</tr>
<tr>
<td>Melon (e.g. watermelon, rockmelon, honeydew melon)</td>
<td>$\chi^2 (4) = 3.81, P = 0.43$</td>
<td>$\chi^2 (4) = 0.27, P = 0.99$</td>
</tr>
<tr>
<td>Pineapple</td>
<td>$\chi^2 (2) = 1.74, P = 0.42$</td>
<td>$\chi^2 (2) = 0.19, P = 0.91$</td>
</tr>
<tr>
<td>Mango</td>
<td>$\chi^2 (2) = 1.17, P = 0.56$</td>
<td>$\chi^2 (2) = 0.32, P = 0.85$</td>
</tr>
<tr>
<td>Chocolate</td>
<td>$\chi^2 (4) = 3.36, P = 0.50$</td>
<td>$\chi^2 (4) = 2.80, P = 0.59$</td>
</tr>
<tr>
<td>Cakes, sweet muffins, scones or pikelets</td>
<td>$\chi^2 (4) = 1.39, P = 0.85$</td>
<td>$\chi^2 (4) = 3.39, P = 0.49$</td>
</tr>
<tr>
<td>Sweet pies or sweet pastries</td>
<td>$\chi^2 (4) = 2.23, P = 0.69$</td>
<td>$\chi^2 (4) = 2.72, P = 0.61$</td>
</tr>
<tr>
<td>Other puddings or desserts</td>
<td>$\chi^2 (4) = 2.99, P = 0.56$</td>
<td>$\chi^2 (4) = 1.80, P = 0.77$</td>
</tr>
<tr>
<td>Plain sweet biscuits</td>
<td>$\chi^2 (4) = 4.53, P = 0.34$</td>
<td>$\chi^2 (4) = 8.42, P = 0.08$</td>
</tr>
<tr>
<td>Cream chocolate biscuits</td>
<td>$\chi^2 (4) = 1.72, P = 0.77$</td>
<td>$\chi^2 (4) = 2.82, P = 0.59$</td>
</tr>
</tbody>
</table>
Table 7.10 *Hedonic ratings (tertile groups) and frequency of reported complex carbohydrate based foods consumption.*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hedonic Ratings of Carbohydrate Solutions</th>
<th>Hedonic Ratings of Carbohydrate Foods</th>
</tr>
</thead>
<tbody>
<tr>
<td>White bread, toast, or rolls (serves consumed within an average month)</td>
<td>$\chi^2(4) = 5.04, P = 0.28$</td>
<td>$\chi^2(4) = 2.77, P = 0.59$</td>
</tr>
<tr>
<td>Wholemeal/mixed grain bread, toast or roll</td>
<td>$\chi^2(4) = 9.59, P = 0.05$</td>
<td>$\chi^2(4) = 2.35, P = 0.67$</td>
</tr>
<tr>
<td>Muesli</td>
<td>$\chi^2(4) = 3.11, P = 0.54$</td>
<td>$\chi^2(4) = 1.82, P = 0.40$</td>
</tr>
<tr>
<td>Breakfast cereal</td>
<td>$\chi^2(4) = 0.89, P = 0.93$</td>
<td>$\chi^2(4) = 2.44, P = 0.65$</td>
</tr>
<tr>
<td>Rice (including white or brown)</td>
<td>$\chi^2(4) = 3.89, P = 0.42$</td>
<td>$\chi^2(4) = 6.93, P = 0.14$</td>
</tr>
<tr>
<td>English muffin, bagel, or crumpet</td>
<td>$\chi^2(2) = 3.06, P = 0.22$</td>
<td>$\chi^2(2) = 0.53, P = 0.97$</td>
</tr>
<tr>
<td>Dry or savoury biscuits</td>
<td>$\chi^2(2) = 1.64, P = 0.44$</td>
<td>$\chi^2(2) = 0.41, P = 0.81$</td>
</tr>
<tr>
<td>Crispbread crackers</td>
<td>$\chi^2(2) = 0.51, P = 0.78$</td>
<td>$\chi^2(2) = 0.51, P = 0.78$</td>
</tr>
<tr>
<td>Cooked porridge</td>
<td>$\chi^2(2) = 0.13, P = 0.94$</td>
<td>$\chi^2(2) = 0.64, P = 0.73$</td>
</tr>
<tr>
<td>Pasta (including filled), and noodles</td>
<td>$\chi^2(2) = 0.12, P = 0.94$</td>
<td>$\chi^2(2) = 0.70, P = 0.70$</td>
</tr>
<tr>
<td>Potato, boiled, mashed, baked</td>
<td>$\chi^2(2) = 1.06, P = 0.59$</td>
<td>$\chi^2(2) = 0.04, P = 0.97$</td>
</tr>
<tr>
<td>Pumpkin</td>
<td>$\chi^2(2) = 1.25, P = 0.54$</td>
<td>$\chi^2(2) = 2.65, P = 0.27$</td>
</tr>
<tr>
<td>Sweet potato</td>
<td>$\chi^2(2) = 3.10, P = 0.21$</td>
<td>$\chi^2(2) = 3.87, P = 0.14$</td>
</tr>
<tr>
<td>Peas</td>
<td>$\chi^2(2) = 1.01, P = 0.61$</td>
<td>$\chi^2(2) = 0.60, P = 0.74$</td>
</tr>
<tr>
<td>Other beans, lentils</td>
<td>$\chi^2(2) = 0.55, P = 0.76$</td>
<td>$\chi^2(2) = 1.35, P = 0.51$</td>
</tr>
<tr>
<td>Hot chips</td>
<td>$\chi^2(4) = 5.39, P = 0.25$</td>
<td>$\chi^2(4) = 0.29, P = 0.99$</td>
</tr>
<tr>
<td>Potato chips, corn chips, Twisties, etc.</td>
<td>$\chi^2(2) = 7.79, P = 0.10$</td>
<td>$\chi^2(2) = 2.76, P = 0.59$</td>
</tr>
<tr>
<td>Cakes, sweet muffins, scones or pikelets</td>
<td>$\chi^2(4) = 1.28, P = 0.87$</td>
<td>$\chi^2(4) = 5.95, P = 0.20$</td>
</tr>
<tr>
<td>Sweet pies or sweet pastries</td>
<td>$\chi^2(4) = 0.92, P = 0.92$</td>
<td>$\chi^2(4) = 8.50, P = 0.08$</td>
</tr>
<tr>
<td>Other puddings or desserts</td>
<td>$\chi^2(4) = 6.64, P = 0.16$</td>
<td>$\chi^2(4) = 7.31, P = 0.12$</td>
</tr>
<tr>
<td>Plain sweet biscuits</td>
<td>$\chi^2(4) = 4.40, P = 0.36$</td>
<td>$\chi^2(4) = 2.51, P = 0.64$</td>
</tr>
<tr>
<td>Cream chocolate biscuits</td>
<td>$\chi^2(4) = 4.42, P = 0.35$</td>
<td>$\chi^2(4) = 1.76, P = 0.78$</td>
</tr>
</tbody>
</table>
7.5 Discussion

The present study focused specifically on individual differences in oral complex carbohydrate sensitivity and the relationship between oral complex carbohydrate sensitivity, liking of complex carbohydrate solutions and prototypical foods, BMI, and consumption of complex carbohydrate based foods in a large convenience sample of adults. All in, our data support the hypothesis that complex carbohydrates are not invisible to the human palate, and can be sensed in the oral cavity even at low concentration levels by human participants. However, the hypothesis where there will be positive associations between oral complex carbohydrate sensitivity (suprathreshold intensity perception), BMI, liking, and consumption of complex carbohydrate based foods was not supported. There were also no associations between liking towards sweet and complex carbohydrate solutions or prototypical foods, and consumption of sweet and complex carbohydrate based foods.

Our findings replicate those of Lapis et al. (18, 19) and recent observations from our own laboratory (detailed in Chapter 5), signifying that humans may perceive complex carbohydrates in the oral cavity; and contest the knowledge where complex carbohydrates have been assumed to be invisible to the human palate system. (6, 330) Consistent with our previous findings (Chapter 5), there was considerable inter-individual variation for oral complex carbohydrate sensitivity among this sample, and individuals were able to be classified as more or less sensitive or as those who experienced high or low intensity to complex carbohydrate across both measures (DT and ST). For example, there were large individual differences in participant’s DT for complex carbohydrate (i.e. concentration required to reach DT for this sample population ranged roughly around 120-fold across the sample population). Similarly, for intensity ratings, there were also large individual variances in terms of participant’s
Chapter 7 – Study 3(a) The Associations between Oral Complex Carbohydrate Sensitivity, BMI, Liking, and Consumption of Complex Carbohydrate Based Foods

perceived complex carbohydrate intensity ratings. For instance, complex carbohydrate (maltodextrin) at the 11.2% w/v concentration was rated as 32 gLMS by a participant who experienced high intensity, but a participant who experienced low intensity rated the same concentration sample as 7 gLMS. Considering the concentrations used, it is possible that the participants detected the complex carbohydrates in the maltodextrin samples instead of the sugars. With the methodologies employed to measure oral complex carbohydrate sensitivity, we are optimistic that the participants’ DT and ST observed for complex carbohydrate were based on their oral sensitivity to complex carbohydrates, and not on other orosensory clues such as olfaction and visual. That is, in the present study a) a wide range of concentrations was used starting from low concentration levels; b) participants did not swallow any samples during testing, and rinsed their mouth with deionised water between tasting samples; c) participants wore nose clips when tasting samples to reduce any orthonasal and retronasal olfaction clues during testing; d) red lights were turned on during testing in our sensory laboratory to reduce any colour or visual cues when tasting samples; e) food-graded water soluble carbohydrate (maltodextrin) with a low DE was used; and f) samples were prepared fresh on the day. However, we could not rule out the possibility that at higher concentration levels some participants may be able to detect differences between samples due to texture/viscosity. Although inter-individual variation to oral complex carbohydrate sensitivity is large, and there were participants who were able to detect complex carbohydrate in water even at the lowest concentrations tested (0.1% w/v), the evidence is not conclusive that the DT and ST reported for maltodextrin were not due to additional textural cues.

Overall, the current findings indicate that oral complex carbohydrate sensitivity (DT and ST) are not associated with the frequency of consumption of
Chapter 7 – Study 3(a) The Associations between Oral Complex Carbohydrate Sensitivity, BMI, Liking, and Consumption of Complex Carbohydrate Based Foods

complex carbohydrate based foods measured. A possible explanation for the lack of associations between oral complex carbohydrate sensitivity and the consumption of complex carbohydrate based foods in comparison to what we found in the previous chapter (Chapter 6) could be due to differences in the dietary assessment tools used. For example, the present study examined individual foods only whereas the other study examined percentage energy from starch and overall energy intake and not individual foods (Chapter 6). Perhaps, it may be the case that the frequency of intake of carbohydrate based foods is actually unlikely to differ between people who are more or less sensitive or between those who experienced high intensity or low intensity, but rather, what may be going on is that people who are more sensitive or experienced high intensity may consume greater quantities of complex carbohydrate based foods when they are consumed (which we did not assess in this study).

The observation that BMI measurements do not differ between measures of oral complex carbohydrate sensitivity (DT and ST) suggests that BMI is not regulated by oral sensitivity towards complex carbohydrate. Our recent research provides support for this proposition as we have established significant differences in oral complex carbohydrate sensitivity (maltodextrin, oligofructose) amongst participants with smaller and larger waist circumference measurements (Chapter 6). That is, more sensitive participants or those who experienced high intensity towards both complex carbohydrates (maltodextrin, oligofructose) had on average more than 10 cm differences in terms of waist circumference measurements in comparison to less sensitive participants or those who experienced low intensity (Chapter 6). These differences were, however, not found for BMI measurements (Chapter 6), suggesting that oral sensitivity towards complex carbohydrate (maltodextrin) is associated with excess intra-abdominal fat mass specifically but not weight and height measurements.
Although BMI measurements are widely used for the evaluation of obesity both in adults, (353, 354) and children, (356, 366) body fat distribution and abdominal fat mass can vary substantially across populations and can differ greatly within a narrow range of BMI. (367)

In regards to sweet taste, although a range of DTs and STs were observed for glucose, there were no significant differences between sweet taste function, BMI, and consumption frequency of the sweet foods measured. While the role of taste sensitivity in promoting intake of specific foods or ingredients associated with obesity has been a long-investigated area of interest, the present findings were consistent with a large body of evidence indicating no significant associations between sweet taste function, BMI, and dietary intake. (158, 189-196, 299)

The present study found no significant differences in terms of sweet liking (both solutions and prototypical foods), body weight, and frequency of consumption of sweet foods. Previous data investigating the link between sweetness liking, BMI, and intake of sweet foods is conflicting, with most data failing to find any significant associations. (177, 190, 192, 194, 195, 197-199) Similarly, at present, we also found no significant differences in terms of complex carbohydrate liking (solutions and prototypical foods), BMI, and frequency of consumption of complex carbohydrate based foods. The present findings are interesting as animal models provide robust evidence to suggest a link between hedonic response and intake of complex carbohydrate solutions, as animals find complex carbohydrate solutions to be more palatable than sugars solutions at low concentrations in two bottle tests, (17) and can condition strong and long-lasting flavour preferences. (349) In contrast, available data from a brain study investigating how complex carbohydrate intake affects liking and wanting task-related signalling found no significant effect of BMI on liking and
Chapter 7 – Study 3(a) The Associations between Oral Complex Carbohydrate Sensitivity, BMI, Liking, and Consumption of Complex Carbohydrate Based Foods wanting task-related signalling for complex carbohydrates. (368) Interestingly, an inverse relationship was observed between liking task-related signalling after a complex carbohydrate-rich meal and intake of complex carbohydrate foods (i.e. liking reduces after complex carbohydrate intake). (368) Nevertheless, such discrepancies in findings should not be surprising, as actual intake of real food could be caused by a combination of many different factors ranging from molecular biology level to environmental factors such as income and cultural factors. (321) Furthermore, one could argue that liking of tastant solutions or prototypical foods bears little relevance of actual intake of real food in everyday life. Therefore, it has proved difficult to link adult taste liking with BMI and diet, whether in a real-world or laboratory setting. (319)

This study needs to be considered alongside limitations, which may have confounded the results. First, participants within the study may be more health and nutrition conscious, thus restricting the generalisability of the results, as they were food and nutrition university students. (369) In addition, the majority of participants in the current study were females within the normal BMI range, which makes it difficult to generalise results to the broader young adult population.
Chapter 7 – Study 3(a) The Associations between Oral Complex Carbohydrate Sensitivity, BMI, Liking, and Consumption of Complex Carbohydrate Based Foods

7.6 Conclusions

There are large inter-individual variations in oral complex carbohydrate sensitivity measures (DT and RT), and participants could be grouped into more sensitive and less sensitive or those who experienced high and low intensity groups for complex carbohydrate (maltodextrin). No significant differences were found between oral complex carbohydrate sensitivity, BMI, and consumption frequency of complex carbohydrate based foods measured. Similarly, no differences were observed between liking of sweet and complex carbohydrates solutions or prototypical foods, BMI, and food intake. All in, these results provide strong support for the proposition that complex carbohydrates are not invisible to the human palate, and can be sensed in the oral cavity even at low concentration levels in a large sample group.
Chapter Eight: Study 3(b) The Associations between Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, Liking, and Consumption of Ad Libitum Sweet and Complex Carbohydrate Milkshakes

8.1 Introduction

It is thought that a person’s ability to taste or sense taste qualities in the oral cavity (initial process of taste perception) is one of the many factors influencing food acceptance, and as such, the ability to taste may play an essential role in modulating energy intake. (33) Aside from the five basic tastes (sweet, sour, salty, bitter, umami) and much new evidence supporting a sixth taste response for fatty acids, (55-65) more recent psychophysics literature has shown that humans were able to taste complex carbohydrates (maltodextrin, glucose oligomer) and that the sweetness perception from simple carbohydrates were found to be independent from the complex carbohydrates measured. (18, 19) Similarly, a more recent study from our laboratory (Chapter 5) established that humans were able to detect complex carbohydrates (maltodextrin, oligofructose) consistently in water. Furthermore, individual’s abilities to perceive these complex carbohydrates were significantly different from the universally accepted five basic taste qualities (perceptual independence with sweet taste is at detection threshold only, but overlap with sweetness at intensity) (Chapter 5), proposing the potential existence of an unidentified complex carbohydrate taste receptor in humans that responds to complex
carbohydrates independently of those of simple carbohydrates at low concentration levels. (8)

However, in order for oral perception of complex carbohydrates to be classified as a taste component, taste receptors have to be identified, potential discrimination based on texture must be conclusively addressed, and the potential physiological implication of taste perception has to be investigated.

Excess energy intake is recognised as a strong contributing factor to the global rise of being overweight and obese. (31, 303) The prevalence of obesity worldwide has been increasing over the past years, necessitating an increased understanding of the drivers of food intake. Foods high in dietary carbohydrates in the form of complex carbohydrates and simple carbohydrates represent a major source of energy in our diet. For example, the estimated Acceptable Macronutrient Distribution Ranges (AMDR) related to reduced risk of chronic disease are 45-65% of total energy intake from carbohydrate, 20-35% from fat, and 15-25% from protein. (370) Aside from some fruits, there is much less sugar in plant food sources (e.g. tubers, legumes, grains) in comparison to complex carbohydrates, but it is the simple carbohydrates (sugars) with their hedonically palatable sweet taste that are the most sought after carbohydrate. (2) Foods high in dietary carbohydrate (simple carbohydrate, complex carbohydrate) have been shown to have a weaker effect on satiation in comparison to other food groups such as those high in dietary protein (257, 258) and result in overconsumption of energy due to their palatability. (259)

Individual differences in their ability to perceive complex carbohydrates and the role of oral complex carbohydrate sensitivity in the overconsumption of energy or specific foods associated with the development of obesity deserve more attention. For example, individuals vary in terms of their satiety responses to dietary fat, (365, 371, 372) and one
Chapter 8 – Study 3(b) The Associations between Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, Liking, and Consumption of *Ad Libitum* Sweet and Complex Carbohydrate Milkshakes

of the factors influencing the satiating responses to dietary fat appears to be due to the individual’s oral and gastrointestinal sensitivity to fatty acids. (256, 365) It has been suggested that abnormalities in any or several nutrient receptors are known to influence intake of specific food components related to the nutrient receptor. (253) For example, it has been well documented in the literature that an individual’s abilities to detect bitter tastants at low concentrations (*i.e.* n-6-propylthiouracil (PROP) and phenylthiocarbamide (PTC)) are determined via genetics, (254) and influence the palatability and consumption of bitter-tasting vegetables such as kale, broccoli, and Brussels sprouts. (253) This behaviour has also been reported for orally detected compounds such as fatty acids, whereby a negative relationship between habitual fat intake and oral sensitivity to fatty acids has been found, that is, individuals who were less sensitive to fatty acids were found to consume more fatty foods. (55, 365) Similarly, after restricting dietary fat intake over a four-week period (57) and six-week period, (60) changes in oral sensitivity to fatty acids have also been seen (*i.e.* more sensitive to fatty acids following decreased intake of dietary fats). A recent study found that oral sensitivity to fatty acids was negatively associated with *ad libitum* intake of high-fat meals (*i.e.* satiation or intrameal satiety in response to fat; (256)), and in subsequent meal intake (*i.e.* satiety responses to fat; (257)). In regards to oral complex carbohydrate sensitivity, a recent cross-sectional study from our laboratory observed a positive association between oral complex carbohydrate sensitivity, intake of complex carbohydrate foods (assessed via 4-day diet diary and Food Frequency Questionnaire), as well as waist measurements (*i.e.* being more sensitive/experienced high intensity to complex carbohydrate was associated with greater energy and starch intakes and a bigger waist measurement) (*Chapter 6*). However, as the previous study used self-
reported dietary measures of habitual/usual intake, it is unclear if the differences in dietary intake were solely due to taste as consumption of foods in the real world, a much less controlled environment than a laboratory, could be influenced by many different factors. (303) It is therefore important to understand whether oral complex carbohydrate sensitivity influences satiation (i.e. meal size or intrameal satiety) from dietary carbohydrate using an experimental approach in controlled laboratory conditions to look at food or energy intake. If there is an effect of complex carbohydrate on satiation, it is unclear whether individual’s liking of complex carbohydrate foods influences this effect as foods with higher palatability could trigger overeating. (373)

8.2 Aims, Hypotheses, and Terminologies

8.2.1 Aims

The aim of this chapter was to investigate if oral sensitivity to complex carbohydrate relates to ad libitum consumption of complex carbohydrate foods in a sample group of adults. We assessed this by comparing homogenous milkshakes with a sweet and a non-sweet carbohydrate. A secondary aim was to investigate if liking towards complex carbohydrate foods plays a role in ad libitum intake of complex carbohydrate foods.
8.2.2 Hypotheses

- Oral suprathreshold intensity perception for complex carbohydrate will be positively associated with *ad libitum* consumption of complex carbohydrate based foods, but detection threshold for complex carbohydrate will not be associated with *ad libitum* consumption of complex carbohydrate based foods;
- Liking towards complex carbohydrate foods will be positively associated with *ad libitum* consumption of complex carbohydrate based foods.

8.2.3 Terminologies

Please refer to Section 7.2.3.

8.3 Subjects, Materials, and Methods

8.3.1 Subjects

According to previous literature, (256) a difference of 10% in intake (in g) would be detected using 49 participants in a paired design with the following assumptions: $\alpha = 0.05$, 2-sided, power of 80%, and a variation of 25%. Participants were recruited from a convenience sample of 138 students enrolled in a third-year Sensory Evaluation of Foods unit during March 2016 at Deakin University, Melbourne campus, Australia. A total of 132 participants gave written informed consent to take part in the study (response rate = 96%). Data were excluded for individuals who: (1) were smokers ($n = 8$); (2) were pregnant or lactating ($n = 3$); (3) were taking any prescription medication that may
interfere with their ability to taste \((n = 3)\); or (4) had a history of any food allergy that may interfere with the study \((i.e.\) fructan, gluten, and lactose intolerance, \(n = 21)\). Eight participants were also excluded from this study, as they did not attend all of the sessions. A dietary restraint score was measured according to factor one of the Three-Factor Eating Questionnaire. The mean ± SD restraint score was 8.9 ± 3.7 and 33 participants were identified as restrained eaters (defined by a score of > 11 on factor one of the Three-Factor Eating Questionnaire), and were removed from the analyses (Figure 8.1). (1) Participants were asked to abstain from eating and drinking (except room temperature water) for two hours prior to each session. This study was approved by the institutional review board regulations of Deakin University (2012_162). The experimental protocol was also registered under the Australian New Zealand Clinical Trials Registry (ACTRN12617000551392), www.anzctr.org.au. This study also complies with the Declaration of Helsinki for Medical Research involving Human Subjects.

### 8.3.2 Study Design

Participants consumed two different iso-caloric preload milkshakes followed by \textit{ad libitum} intake of milkshakes – 1) sweet milkshake (glucose), 2) complex carbohydrate milkshake (maltodextrin) in a randomised crossover design. Participants attended two laboratory sessions, separated by at least seven days of wash out period. The outlines of the two sessions are shown in Figure 8.2.
Chapter 8 – Study 3(b) The Associations between Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, Liking, and Consumption of *Ad Libitum* Sweet and Complex Carbohydrate Milkshakes

*Figure 8.1* Number of participants who were recruited, screened, and completed both sessions. The dietary restraint score was measured according to factor one of the Three-Factor Eating Questionnaire. (1) Restrained eaters were defined as participants with a score on factor one of > 11 on the Three-Factor Eating Questionnaire.

- 138 potential participants were assessed for eligibility
  - 6 decided not to participate
- 132 participants participated
  - 35 participants were ineligible: 8 = smokers, 3 = pregnant/lactating, 3 = medications, 21 = food allergies
  - 8 did not attend all sessions
- 89 participants completed both sessions
  - 33 participants identified as restrained eaters
- 56 participants included in analyses

236
Figure 8.2 The study outline. The left chart represents the session outline for session one, middle chart represents the session outline for session two, and the right chart represents the online questionnaires. Each session lasted about two hours. As the data collection was part of a laboratory class, participants were given intermittent breaks (teaching) lasting 15-30 minutes between each task.
Chapter 8 – Study 3(b) The Associations between Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, Liking, and Consumption of *Ad Libitum* Sweet and Complex Carbohydrate Milkshakes

As the sessions were part of a laboratory class, each class (7 participants maximum at a time) was randomly assigned to the sequence of sweet and complex carbohydrate milkshakes using a web-based program ([http://randomizer.org](http://randomizer.org)). In addition, during the same sessions, detection threshold (DT) and suprathreshold intensity perception (ST) for glucose (sweet) and maltodextrin (complex carbohydrate), hedonic ratings for glucose and maltodextrin solutions, and hedonic ratings for a range of sweet and complex carbohydrate prototypical foods were also determined. Each session lasted about 2 hours, and participants were given breaks between tasks lasting 15-30 minutes.

Demographic information was also collected, including sex, age, height, and weight measurements. Body mass index (BMI, kg/m²) was calculated from the height and weight measurements. Participants also completed three online questionnaires: Food Frequency Questionnaire (FFQ), Likes and Dislikes Questionnaire, and a Three-Factor Eating Questionnaire within 1 week of sensory testing. Psychophysics tasks (DT, ST), consumption of milkshakes, as well as hedonic ratings for a range of sweet and complex carbohydrate solutions were conducted in computerised, partitioned sensory booths in the Centre for Advanced Sensory Science using Compusense Cloud Software as part of the Compusense Academic Consortium (Compusense Inc., Ontario, Canada). Hedonic ratings for a range of sweet and complex carbohydrate based foods were conducted in individual workbenches at our teaching laboratory. Filtered deionised water was used as an oral rinsing agent. Participants were instructed to rinse their mouths with filtered deionised water for five seconds before beginning each task and between each sample set (except in between drinking preload milkshakes and *ad libitum* milkshakes). To eliminate any visual or olfactory input, all psychophysics tasks were conducted under red lighting, and
participants were asked to wear nose clips during testing. All solutions and prototypical foods were served at room temperature, with a three-digit code allocated to each sample. Milkshakes were served chilled at ± 3°C.

8.3.3 Participant training

Prior to using the general Labeled Magnitude Scale (gLMS) to rate taste intensity, participants were trained using the standard protocol outlined by Green et al. (266, 267) except the top of the scale was described as the strongest imaginable sensation of any kind (Appendix G, described in Chapter 2). (75) The 100-point scale comprised the following adjectives: ‘no sensation’ = 0, ‘barely detectable’ = 1.5, ‘weak’ = 6, ‘moderate’ =17, ‘strong’ = 35, ‘very strong’ = 52, and ‘strongest imaginable’ = 100 (Bartoshuk, 2000). Scales with only adjectives (not numbers) were presented to participants. During the training session, participants were taught to rate the intensity of the perceived sensation relative to a remembered or imagined sensation when using the gLMS scale. Participants were required to rate a list of seven remembered or imagined sensations, such as the warmth of the lukewarm water, the pain from biting of the tongue, and the sweetness of fairy floss (known as cotton candy in the USA, or candy floss in the UK).
8.3.4 Stimuli and Test Foods

Glucose was used to investigate sweet taste function (DT and ST for sweet taste; for details of stimuli see Table 8.1; detailed in Chapter 2). Maltodextrin was used to investigate oral complex carbohydrate sensitivity (DT and ST for complex carbohydrate). Maltodextrin with a dextrose equivalent (DE) of five was used in this study as it contains the lowest possible amount of free sugars (glucose, maltose) yet is soluble in water. DE is a measure of the percentage of reducing sugars relative to glucose on a dry basis. (269)

All solutions were prepared fresh on the day of testing using filtered deionised water (Cuno Filter Systems FS117S, Meriden, CT, USA) and stored in glass beakers at room temperature (20 ± 1 ºC).

The sweet (glucose) and complex carbohydrate (maltodextrin) milkshakes (per 100g) consisted of: 8.8% w/w glucose/maltodextrin (The Melbourne Food Depot, Melbourne, Australia; Star-Dri 5, Tate & Lyle Ingredients Americas, USA), 63.7% w/w long-life skim milk (99.9% fat free; Devondale Murray Goulburn, Melbourne, Australia), 26.5% w/w light thickened cream (~18% fat; Bulla, Derrimut, Australia), and 1.0% w/w imitation vanilla essence (Queen Fine Foods, Alderley, Australia). The nutrient compositions of the milkshakes were calculated using the Foodworks8 (Xyris Software) (Table 8.2). The milkshakes were mixed until no lumps were visible using an immersion (stick) blender for 15 seconds (per 100g) at 10,000 rpm (KitchenAid KHB2569 Hand Blender, Whirlpool Corporation, Michigan, USA). All milkshakes were prepared fresh on the day of testing and stored refrigerated (± 3ºC) using plastic food storage containers.
Table 8.1 *Sweetener and complex carbohydrate concentrations used for determination of detection thresholds.*

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Concentration (% w/v)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td></td>
<td>0.05</td>
<td>0.09</td>
<td>0.1</td>
<td>0.2</td>
<td>0.4</td>
<td>0.6</td>
<td>1.1</td>
<td>1.8</td>
<td>2.9</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td></td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.6</td>
<td>1.1</td>
<td>1.9</td>
<td>3.6</td>
<td>6.3</td>
<td>11.2</td>
</tr>
</tbody>
</table>

*Amount of Glucose in Maltodextrin (x10^3)*

|                           | 0.9 | 1.8 | 2.7 | 5.4 | 9.9 | 17.1 | 32.4 | 56.7 | 100.8 |

*Amount of Total Sugars in Maltodextrin (x10^3)*

|                           | 1.7 | 3.4 | 5.1 | 10.2 | 18.7 | 32.3 | 61.2 | 107.1 | 190.4 |

The concentration series for glucose and maltodextrin were prepared with successive 0.25 log dilution steps. Reference chemical details: glucose (The Melbourne Food Depot, Melbourne, Australia); maltodextrin (Star-Dri 5, Tate & Lyle Ingredients Americas, USA). The ninth concentration was presented only when participants were unable to detect a difference from water solution in the previous eight. (70) Calculation of the amount of common and total sugars in maltodextrin concentrations were according to the report of analysis by the Australian Government National Measurement Institute from samples used in this study, where there were a total of 1.7g/100g (1.7% w/w) of free sugars for the maltodextrin (Glucose: 0.9% w/w).
Table 8.2 *Nutrient composition of sweet and complex carbohydrate milkshakes containing different amount of glucose and maltodextrin.*

<table>
<thead>
<tr>
<th></th>
<th>Sweet Milkshake</th>
<th>Complex Carbohydrate Milkshake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Per 100g</td>
<td>Per 100g</td>
</tr>
<tr>
<td>Energy, kJ</td>
<td>454.3</td>
<td>440.7</td>
</tr>
<tr>
<td>Carbohydrate, g</td>
<td>13.2</td>
<td>11.8</td>
</tr>
<tr>
<td>Sugars, g</td>
<td>12.8</td>
<td>4.5</td>
</tr>
<tr>
<td>Starch, g</td>
<td>0.4</td>
<td>7.3</td>
</tr>
<tr>
<td>Protein, g</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>Fat, g</td>
<td>5.3</td>
<td>5.3</td>
</tr>
</tbody>
</table>

The nutrient composition of the milkshakes (8.8% w/w glucose/maltodextrin, 63.7% w/w long-life skim milk, 26.5% w/w light thickened cream, and 1% w/w imitation vanilla essence) per 100g was calculated using Foodworks8 (Xyris Software).

### 8.3.4.1 Analysis of Common Sugars in Maltodextrin Sample

To determine if the maltodextrin used in this study would be a suitable product, four percent w/v maltodextrin solutions were prepared for High Performance Liquid Chromatography (HPLC). The complex carbohydrate extracts were clarified with 25mL acetonitrile and filtered through a 0.45um filter into a 2mL vial. To determine the amount of common sugars in samples, filtered solutions were analysed by HPLC using amino column with an acetonitrile/water mobile phase containing salt and refractive index detection. Quantitation was made using a standard solution containing known amount of fructose, glucose, sucrose, maltose and lactose. Samples were measured in duplicate.

There were a total of 1.7g/100g (1.7% w/w) of common sugars for the maltodextrin used in this study (Glucose: 0.9% w/w) (Table 8.3). Detailed in Table 8.1 are the amount of glucose and total sugars (% w/v) present in each maltodextrin DT concentration.

Table 8.3 *Common sugars composition of the maltodextrin used in the present study.*

<table>
<thead>
<tr>
<th>Proximates</th>
<th>Maltodextrin (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0.9</td>
</tr>
</tbody>
</table>
Chapter 8 – Study 3(b) The Associations between Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, Liking, and Consumption of Ad Libitum Sweet and Complex Carbohydrate Milkshakes

<table>
<thead>
<tr>
<th>Fructose</th>
<th>&lt;0.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>Maltose</td>
<td>0.8</td>
</tr>
<tr>
<td>Lactose</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>Total Sugars</td>
<td>1.7</td>
</tr>
</tbody>
</table>

These analyses were determined by the Australian Government National Measurement Institute, and were conducted by High Performance Liquid Chromatography (HPLC). 20g of each sample were sent for analyses.

8.3.5 Detection Threshold Determination for Sweet Taste and Oral Sensitivity to Complex Carbohydrates

DT was determined using the procedure outlined in the International Standards Organisation (ISO) Method of Investigating Sensitivity of Taste (described in Chapter 2). (265) Table 8.1 gives the nine concentrations used to assess DT for sweet taste and oral complex carbohydrate sensitivity (the ninth concentration being presented only when participants were unable to detect a difference from water solution in the previous eight). (265) The concentration series for glucose and maltodextrin were prepared with successive 0.25 log dilution steps. (299) Concentration for maltodextrin was derived based on previous published findings of perceptually distinctive taste sensation concentration (i.e. see Lapis et al. (18) and systematic literature review by e Silva et al. (289)) without perceivable viscosity. After pilot testing, a concentration range between 0.11-11.2 percent (w/v) was used to measure DT levels for maltodextrin.

The eight samples for each stimulus were served in ascending concentration (15 mL per sample, in accordance with the standard ISO method), and each stimulus was presented to participants independently. Participants were unaware of the presentation order. Participants were instructed to taste each sample for five seconds then spit and rate...
whether: there was an absence of taste/oral perception (water-like); or if a taste/oral perception was identified but not recognised. (261) DT was defined as the concentration at which the participants selected the ‘taste/oral perception identified, but unknown taste quality/oral perception’. (261)

### 8.3.6 Suprathreshold Intensity Ratings for Glucose and Maltodextrin

Three concentrations (weak, medium, and strong) and a control (blank) solution were prepared to determine perceived suprathreshold intensity for glucose and maltodextrin, as described in Chapter 2 (Table 8.4). These concentrations were derived through informal bench-top testing (ascending intensity). The concentrations for each stimulus ranged from ‘weak’ to ‘strong’ on the gLMS. These samples were presented to participants in a randomised order.
Table 8.4 Concentrations (weak, medium, and strong intensity) of glucose and maltodextrin used for determination of suprathreshold intensity.

<table>
<thead>
<tr>
<th></th>
<th>Concentration (% w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weak</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.3</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>3.6</td>
</tr>
<tr>
<td>Amount of Glucose in Maltodextrin (x10^{-3})</td>
<td>32.4</td>
</tr>
<tr>
<td>Amount of Total Sugars in Maltodextrin (x10^{-3})</td>
<td>61.2</td>
</tr>
</tbody>
</table>

Calculation of the amount of common and total sugars in maltodextrin concentrations were according to the report of analysis by the Australian Government National Measurement Institute from samples used in this study, where there were a total of 1.7g/100g (1.7% w/w) of free sugars for the maltodextrin (Glucose: 0.9% w/w).

8.3.7 Standardisation of gLMS Usage with Weight Ratings

To standardise gLMS usage within participants, a modified version of the method used by Delwiche et al. (268) was adapted for this study (Chapter 2). To control for idiosyncratic scale usage, participants were asked to rate the heaviness of six visually identical weights (opaque bottles filled with sand and stone and completely wrapped in aluminium foil; weights of 53, 251, 499, 724, 897, and 1127g) in duplicates (Appendix I). Participants were asked to hold out their non-dominant hand palm up, while the experimenter placed the weighted bottle on the palm of the hand. Participants were instructed to rate the heaviness of each weight using the gLMS.

There was a significant correlation between the overall mean sweetness ratings for glucose and overall mean heaviness ratings ($r = 0.38$, $P < 0.01$). Assuming that the intensity ratings of glucose and the heaviness of the bottles were unrelated, the significant correlation indicates that the gLMS ratings were subject to differences in individual scale-use and thus require standardisation across participants. (54, 70, 268) To determine a
personal standardisation factor, the grand mean for heaviness across weight levels and participants was divided by each participant’s average intensity for heaviness. (54) Each individual’s sweet (glucose) taste intensity and sweetness intensity ratings were multiplied by his or her personal standardisation factor for scale-use bias. (54, 268)

8.3.8 Hedonic Ratings for Sweet and Complex Carbohydrate Solutions and Prototypical Foods

To measure liking of glucose and maltodextrin solutions, three concentrations (weak, medium, and strong) and a control (blank) solution were prepared and presented to participants in a randomised order, as discussed in Chapter 2 (Table 8.4). These solutions were identical to the concentrations used to assess suprathreshold intensity ratings for glucose and maltodextrin.

To assess liking of sweet and complex carbohydrate prototypical foods, participants were required to rate liking of 16 food items (eight sweet taste and eight complex carbohydrate based foods). The foods included in testing had approximately equivalent fat per 100g. Participants were given a variety of different sweet and complex carbohydrate based foods representing a range of dietary carbohydrate contents per serve (differences in grams of sugar or starch per 100g), approximately equivalent to the concentrations (% w/v) used to measure suprathreshold intensity ratings for glucose and maltodextrin. Eight small samples (5-20g) per tray were served in a randomised order, and each tray was presented to participants independently. The foods included in testing can be viewed in Table 8.5 (also see Appendix L for photos of foods used).
Chapter 8 – Study 3(b) The Associations between Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, Liking, and Consumption of *Ad Libitum* Sweet and Complex Carbohydrate Milkshakes

Liking of both solutions and foods was measured using a nine-point hedonic scale ranging from 1 = dislike extremely to 9 = like extremely *(Appendix M)*. All liking evaluations were conducted without the use of nose clips and following psychophysics tests. All solutions/foods were ingested.

### 8.3.9 Standardisation of Hedonic Scale Usage with Non-Food Items

To control for idiosyncratic scale usage, participants were asked to complete a Likes and Dislikes Questionnaire *(Appendix N)*, (276) as described in Chapter 2. Participants were required to rate, on average, how much they liked or disliked a number of food/beverage items and common experiences across ten categories (77 items; *i.e.* grains/cereals, meat/meat alternatives, fast foods, dairy, fruit and vegetables, snack foods, fats/oils, beverages, oral sensation, non-food) on a nine-point hedonic scale. Examples of non-food items (4 items) included how much they liked or disliked jumping in a pool on a hot day, or the glare of headlights.

There was a significant correlation between the overall mean hedonic ratings for food/beverage items and overall mean hedonic ratings for non-food items (*r* = 0.22, *P* < 0.05). As individual hedonic ratings for food/beverage items and non-food items were assumed to be unrelated, the significant correlation indicated that the hedonic scale ratings were subject to differences in individual scale-use and required standardisation across participants.
**Table 8.5** Sweet and complex carbohydrate based foods used for hedonic ratings.

<table>
<thead>
<tr>
<th>Food</th>
<th>Sugar per 100g</th>
<th>Starch per 100g</th>
<th>Fat per 100g</th>
<th>Amount provided (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red kidney beans, unsalted, canned</td>
<td>0.7</td>
<td>13.1</td>
<td>0.5</td>
<td>20g</td>
</tr>
<tr>
<td>Pasta, elbow, boiled, plain</td>
<td>0</td>
<td>28.4</td>
<td>0.3</td>
<td>20g</td>
</tr>
<tr>
<td>Rice, white, boiled with water</td>
<td>0.1</td>
<td>36.0</td>
<td>0</td>
<td>20g</td>
</tr>
<tr>
<td>White bread</td>
<td>3.5</td>
<td>48.0</td>
<td>1.8</td>
<td>20g</td>
</tr>
<tr>
<td>Weet-Bix (Australian breakfast cereal biscuit)</td>
<td>3.3</td>
<td>67.0</td>
<td>1.4</td>
<td>20g</td>
</tr>
<tr>
<td>Pretzel chips, low fat</td>
<td>1.9</td>
<td>72.8</td>
<td>1.6</td>
<td>20g</td>
</tr>
<tr>
<td>Rice cake, thin, plain</td>
<td>0.2</td>
<td>78.0</td>
<td>2.8</td>
<td>20g</td>
</tr>
<tr>
<td>Rice cracker, plain</td>
<td>4.7</td>
<td>81.5</td>
<td>1.1</td>
<td>20g</td>
</tr>
<tr>
<td>Tomato puree (passata), unsalted, canned</td>
<td>8.2</td>
<td>0</td>
<td>1.1</td>
<td>15mL</td>
</tr>
<tr>
<td>Apples, dried</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>20g</td>
</tr>
<tr>
<td>Gelatine dessert, blackcurrant flavoured</td>
<td>19.5</td>
<td>0</td>
<td>0</td>
<td>15g</td>
</tr>
<tr>
<td>Chocolate flavoured syrup, low fat</td>
<td>54.8</td>
<td>0</td>
<td>0.4</td>
<td>15mL</td>
</tr>
<tr>
<td>Strawberry jam</td>
<td>64.0</td>
<td>0</td>
<td>0</td>
<td>15mL</td>
</tr>
<tr>
<td>Raisins</td>
<td>71.4</td>
<td>0</td>
<td>0</td>
<td>20g</td>
</tr>
<tr>
<td>Honey</td>
<td>82.5</td>
<td>0</td>
<td>0</td>
<td>15mL</td>
</tr>
<tr>
<td>Fairy floss (known as cotton candy in the USA, or candy floss in the UK)</td>
<td>97.2</td>
<td>0</td>
<td>0</td>
<td>5g</td>
</tr>
</tbody>
</table>

Food details: Red kidney beans, unsalted, canned (Coles Homebrand, Coles, Australia); Pasta, elbow (Coles Homebrand, Coles, Australia); Rice, white (SunRice, Ricegrowers Limited, Australia); White bread (Coles Homebrand, Coles, Australia); Weet-Bix (Sanitarium Health and Wellbeing Company, Australia); Pretzel chips, low fat (Parker’s, The Smith’s Snackfood Company, Australia); Rice cake, thin, plain (SunRice, Ricegrowers Limited, Australia); Rice cracker, plain (Sakata, The Smith’s Snackfood Company, Australia); Tomato puree (passata), unsalted, canned (Coles Homebrand, Coles, Australia); Apples, dried (Angas Park, Angas Park Fruit Co, Australia); Gelatine dessert, blackcurrant flavoured (Aeroplane Jelly, McCormick & Company, Australia); chocolate flavoured syrup, low fat (Cottee’s, Heinz Foodservice, Australia); Strawberry jam (IXL, SPC Ardmona, Australia); Raisins (Coles Homebrand, Coles, Australia); Honey (Coles Homebrand, Coles, Australia); Fairy floss (The Fairy Floss King, NSW, Australia).
To determine a personal standardisation factor, a similar calculation method with gLMS standardisation was used (i.e. the grand mean for non-food items across all participants was divided by each participant’s average hedonic ratings for non-food items). Each individual’s hedonic ratings were multiplied by his or her personal standardisation factor for scale-use bias.

8.3.10 Satiation Measures - Preload and Ad Libitum Intake of Milkshakes, Drinking Rate, and Appetite and Hedonic Ratings

A modified procedure outlined by Rolls and McDermott (288) was used to assess the satiation effect of sweet and complex carbohydrate milkshakes, as described in Chapter 2. Participants were first served a cup containing 200g of milkshake (sweet: 908.6kJ, complex carbohydrate: 881.4kJ), and were instructed to finish the whole cup of milkshake within a minute (maximum time). Two minutes after consumption of the preload milkshake, participants were presented with another serving of the same milkshake (600g; sweet: 2725.8kJ, complex carbohydrate: 2644.2kJ). For the 600g milkshake, participants were told to drink until they are comfortably full (maximum time: 5 minutes). The serving sizes for preload (200g) and ad libitum (600g) milkshakes were derived through previously published finding by Rolls and McDermott (288) using young adult samples. In that study, (288) a fixed volume of yogurt (300g) was given to participants as a preload as it was found to be the average amount of yogurt consumed by participants. However, as the participants in the present study were mainly young female adults, we chose to use 200g as the serving size for preloads in order to be sure that
participants were given the opportunity to drink until satiated in the \textit{ad libitum} experiment. A concentration of 8.8\% (per 100g of complex carbohydrate milkshake) of maltodextrin was derived based on previous published findings of perceptually distinctive taste sensation concentration without perceivable viscosity. (18, 289) A concentration of 8.8\% (per 100g of sweet milkshake) of glucose was used for sweet milkshakes. During the milkshake experiment, participants were not allowed to drink any water until after the experiment was over.

The \textit{ad libitum} milkshake intake was calculated as the difference in the weight of the cup of milkshake before and after consumption. The milkshake intake in grams was used to determine the energy intake in kilojoules. Drinking rate (g/sec or kJ/sec) was calculated by dividing the \textit{ad libitum} milkshake intake in grams or kilojoules by the total drinking duration (sec). During the milkshake experiment, participants were asked to start drinking the milkshake as soon as they were instructed to start, and to raise their hands quietly to inform the researcher in the room as soon as they had finished. The researcher, using a stopwatch, measured the total duration time (sec) used to drink the \textit{ad libitum} milkshake.

Prior to consuming the preload and \textit{ad libitum} milkshakes, participants completed several questions relating to appetite and hedonic ratings. (213, 256, 290, 291) When the milkshakes were served, participants were instructed to drink a sip of their milkshake and to rate their liking of it on a nine-point hedonic scale. Participants were also instructed to rate their feelings of hunger, fullness, and prospective consumption prior to consumption of both milkshakes (preload and \textit{ad libitum}) on a 100 mm visual analogue scale (VAS)
Chapter 8 – Study 3(b) The Associations between Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, Liking, and Consumption of Ad Libitum Sweet and Complex Carbohydrate Milkshakes

anchored at each end with descriptors (e.g. ‘not hungry at all’ at one end and ‘very hungry’ at the other).

8.3.11 Body Composition

All participants were asked to remove shoes and heavy clothing to ensure accurate measurements, as described in Chapter 2. All body composition measurements were measured first thing during the first session after a 2-hour fast (food only). Participants’ body weight was self-measured to the nearest 0.1 kg using a segmental body composition analyser (TBF-300A) (Tanita Corporation, Tokyo, Japan). Participants’ height was self-measured to the nearest 0.1 cm using a portable stadiometer (Seca213; Seca, Hamburg, Germany). All measurements were repeated twice to ensure accuracy. Averages of measurements for height and weight were used to calculate BMI (weight in kg/m²) and determine weight status (i.e. normal weight or overweight/obese). Weight statuses were defined under World Health Organisation BMI classification. (277)

8.3.12 Statistical analysis

Statistical analysis was performed using IBM SPSS statistical software version 23.0 (SPSS, Chicago, IL, USA). Data are presented as means with standard errors of mean (SEM). Significance was accepted at \( P < 0.05 \). For suprathreshold intensity ratings and hedonic ratings for solutions, the geometric mean score of the three ratings (weak, moderate, and strong) was calculated. For hedonic ratings of a range of sweet and complex carbohydrate foods, a geometric mean score of the eight food items was calculated.
Chapter 8 – Study 3(b) The Associations between Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, Liking, and Consumption of *Ad Libitum* Sweet and Complex Carbohydrate Milkshakes

Descriptive statistics were employed to describe demographic information, sweet taste and oral complex carbohydrate sensitivity thresholds, perceived intensity of sweet taste and oral complex carbohydrate sensitivity, hedonic ratings of sweet taste and complex carbohydrate solutions, prototypical foods, and milkshakes, intake of milkshakes (grams and kJ), drinking rate, appetite ratings, and BMI. DTs for glucose and maltodextrin were treated as grouping variables (tertiles) with participants categorised as more sensitive (1/3), normal sensitive (2/3), and less sensitive (3/3) to explore differences between continuous (milkshake intake, BMI) variables. STs for glucose and maltodextrin were treated as grouping variables (tertiles) with participants categorised as those who experienced low intensity (1/3), moderate intensity (2/3), and high intensity (3/3) to explore differences between continuous (milkshake intake, BMI) variables. DTs and STs for glucose and maltodextrin were grouped into tertiles to allow comparison of most and least sensitive groupings or those groups who experienced low and high intensity (*i.e.* four sets of tertiles were determined: one for DT for glucose and maltodextrin, and one for ST for glucose and maltodextrin). (299) Similarly, individuals’ hedonic ratings for sweet and complex carbohydrate solutions and prototypical foods were treated as grouping variables (tertiles) with participants categorised as those who rated low (1/3), moderate (2/3) and high (3/3) on the hedonic scale to explore differences between variables (milkshake intake). Hedonic ratings for sweet and complex carbohydrate solutions and prototypical foods were grouped into tertiles to allow comparison of those groups who rated low and high on the hedonic scale (*i.e.* four sets of tertiles were determined for hedonic ratings: for sweet solutions, sweet prototypical foods, complex carbohydrate solutions, and complex carbohydrate prototypical foods). An independent *t*-test was used to detect differences in
milkshake intake between more sensitive and less sensitive participants or those who experienced low and high intensity (low and high tertile groups). An independent \( t \)-test was used to detect differences in milkshake intake between those who rated low and high on the hedonic scale groups (low and high tertile groups). Pearson’s product-moment correlations were conducted to also analyse the relationship between sweet taste function and oral complex carbohydrate sensitivity (DTs and STs for glucose and maltodextrin), hedonic ratings for sweet and complex carbohydrate solutions and prototypical foods, milkshakes, and BMI. Appetite ratings and hedonic ratings for milkshakes from before compared with after preload within a session were assessed using paired \( t \) tests. Effects of simple carbohydrate and complex carbohydrate on \textit{ad libitum} milkshake intake, drinking rate, and liking of milkshakes were compared using paired \( t \) tests. The effects of simple carbohydrate and complex carbohydrate on delta appetite ratings and liking of milkshakes (before \textit{ad libitum} intake – rating before preload intake) were compared using paired \( t \) tests. Independent \( t \)-tests were used to analyse differences in terms of sex, order of presentation, and BMI groups between sweet taste function and oral complex carbohydrate sensitivity (DTs and STs for glucose and maltodextrin), liking, and milkshake intake.
8.4 Results

8.4.1 Participants

Of the 56 participants who completed the study, five were males [age 22.6 ± 0.2 years (range 22 – 23 years), BMI 25.3 ± 1.1 kg/m² (range 22.7 – 29.0 kg/m²], and 51 were females [age 23.0 ± 0.6 years (range 20.0 – 41.0 years), BMI 22.1 ± 0.3 kg/m² (range 18.5 – 29.1 kg/m²)]. Out of the total 56 participants, ten were classified as overweight/obese [2 male, 8 female, BMI 26.5 ± 0.4 kg/m² (range 25.2– 29.1 kg/m²)].

8.4.2 Ad Libitum Intake of Sweet and Complex Carbohydrate Milkshakes

The sweet milkshake preload (200g) resulted in 32% greater ad libitum milkshake intake ($P = 0.02$) and energy intake ($P = 0.04$) in comparison with the complex carbohydrate milkshake (Figure 8.3). There were no significant differences in ad libitum consumption of sweet and complex carbohydrate milkshakes found between lean and overweight/obese participants (all $P > 0.05$). Similarly, no significant differences between sex groups and the order of presentation (presented with sweet milkshakes first versus complex carbohydrate milkshakes) in ad libitum consumption of milkshakes were found (all $P > 0.05$).
Chapter 8 – Study 3(b) The Associations between Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, Liking, and Consumption of *Ad Libitum* Sweet and Complex Carbohydrate Milkshakes

*Figure 8.3* Mean ± SEM *ad libitum* milkshake intakes by weight (g) (A) and energy (kJ) (B) of fifty-six adults (*n* = 56) who consumed sweet and complex carbohydrate milkshakes in random order.

* * * * P < 0.05

(A)  

![Bar chart showing milkshake intake by weight (g) for sweet and complex carbohydrate milkshakes.](image)

(B)  

![Bar chart showing energy intake (kJ) for sweet and complex carbohydrate milkshakes.](image)
8.4.3 Liking of Milkshakes, Drinking Rate, and BMI

Liking ratings of preload and _ad libitum_ milkshakes also showed a significantly higher liking rating for the sweet milkshake than for the complex carbohydrate milkshake (all $P < 0.01$) (Figure 8.4). Following preload consumption of each milkshake, liking ratings decreased significantly for _ad libitum_ milkshake (all $P < 0.01$; Table 8.6). There were no significant differences between both types of milkshakes on decrease in liking (delta) ($P > 0.05$). _Ad libitum_ drinking rate expressed as g/sec and kJ/sec did not differ between types of milkshakes (all $P > 0.05$; Table 8.7).

_Ad libitum_ intake of complex carbohydrate milkshake was positively correlated with drinking rate (g/sec; $r = 0.28; P < 0.05$). However, no significant correlations were observed between _ad libitum_ intake of sweet milkshake and drinking rate ($P > 0.05$). Sweet, but not complex carbohydrate milkshake intake was significantly correlated with liking ratings [$r = 0.35 (P < 0.01)$ and $r = 0.06 (P > 0.05)$ for the sweet and complex carbohydrate milkshakes, respectively]. No significant correlations were observed between drinking rate (g/sec) and liking ratings (_ad libitum_) (all $P > 0.05$); however, a significant negative relationship was observed between drinking rate (g/sec) and changes in liking rating for sweet milkshake (delta) ($r = -0.28, P < 0.05$). No significant correlations were observed between BMI and _ad libitum_ intake of both milkshakes (all $P > 0.05$). Similarly, BMI was not significantly correlated with intake differences (delta) of both types of milkshakes ($P > 0.05$). Drinking rates (g/sec), liking ratings (preload and _ad libitum_), and changes in liking ratings for both types of milkshakes (delta) were not correlated with BMI (all $P > 0.05$).
Chapter 8 – Study 3(b) The Associations between Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, Liking, and Consumption of *Ad Libitum* Sweet and Complex Carbohydrate Milkshakes

*Figure 8.4* Mean ± SEM hedonic ratings for preload and *ad libitum* sweet and carbohydrate milkshakes (*n* = 56). The y-axis is the adjusted hedonic ratings from a nine-point hedonic scale. The x-axis represents the preload and *ad libitum* milkshakes measured.

- Preload
- *Ad Libitum*

![Graph showing mean ± SEM hedonic ratings for preload and ad libitum sweet and carbohydrate milkshakes. The y-axis represents the adjusted hedonic ratings from a nine-point hedonic scale. The x-axis represents the preload and ad libitum milkshakes measured.](image)
Table 8.6 *Hedonic ratings and appetite ratings of fifty-six (n = 56) adults who consumed two types of milkshakes containing different amounts of glucose (sweet milkshake) and maltodextrin (complex carbohydrate milkshake) on two separate days.*

<table>
<thead>
<tr>
<th></th>
<th>Sweet milkshake</th>
<th><strong>P</strong>&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Complex carbohydrate milkshake</th>
<th><strong>P</strong>&lt;sup&gt;2&lt;/sup&gt;</th>
<th><strong>P</strong>&lt;sup&gt;3&lt;/sup&gt;</th>
<th><strong>P</strong>&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hedonic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before preload intake</td>
<td>5.9 ± 0.2</td>
<td></td>
<td>4.9 ± 0.3</td>
<td></td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Before <em>ad libitum</em> intake</td>
<td>5.1 ± 0.3</td>
<td>&lt;0.001</td>
<td>4.2 ± 0.2</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ</td>
<td>-0.8 ± 0.2</td>
<td></td>
<td>-0.7 ± 0.2</td>
<td></td>
<td></td>
<td>0.82</td>
</tr>
<tr>
<td><strong>Hunger, mm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before preload intake</td>
<td>59.2 ± 3.2</td>
<td></td>
<td>55.6 ± 3.1</td>
<td>0.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before <em>ad libitum</em> intake</td>
<td>35.9 ± 2.9</td>
<td>&lt;0.001</td>
<td>35.3 ± 3.3</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ</td>
<td>-23.3 ± 2.5</td>
<td></td>
<td>-20.3 ± 2.5</td>
<td></td>
<td></td>
<td>0.30</td>
</tr>
<tr>
<td><strong>Fullness, mm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before preload intake</td>
<td>25.1 ± 2.7</td>
<td></td>
<td>25.4 ± 2.6</td>
<td>0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before <em>ad libitum</em> intake</td>
<td>54.5 ± 2.9</td>
<td>&lt;0.001</td>
<td>55.8 ± 3.2</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ</td>
<td>29.5 ± 2.7</td>
<td></td>
<td>30.4 ± 2.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Prospective consumption, mm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>Before preload intake</td>
<td>59.8 ± 3.2</td>
<td></td>
<td>58.7 ± 2.6</td>
<td>0.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before <em>ad libitum</em> intake</td>
<td>39.2 ± 3.2</td>
<td>&lt;0.001</td>
<td>37.8 ± 3.2</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ</td>
<td>-20.6 ± 3.2</td>
<td></td>
<td>-20.9 ± 2.6</td>
<td></td>
<td></td>
<td>0.91</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are means ± SEMs. Δ: rating before *ad libitum* intake—rating before preload intake. Hedonic values are adjusted hedonic ratings from a nine-point hedonic scale. *P* < 0.05.

<sup>2</sup>*P* values representing differences between before preload intake and before *ad libitum* intake in hedonic, hunger, fullness, and prospective consumption ratings (paired t tests).

<sup>3</sup>*P* values representing differences between sweet and complex carbohydrate milkshake sessions before preload intake in hedonic, hunger, fullness, and prospective consumption ratings (paired t tests).

<sup>4</sup>*P* values representing differences between sweet and complex carbohydrate milkshakes in terms of changes before preload intake and before *ad libitum* intake (delta) in hedonic, hunger, fullness, and prospective consumption ratings (paired t tests).
8.4.4 Appetite Ratings

No significant differences were observed between ratings of fullness, hunger, and prospective consumption before consumption of preload milkshakes (all $P > 0.05$; Table 8.6), signifying that participants were in a similar state of satiety before preload intake. Fullness ratings increased, hunger decreased, and ratings of prospective consumption decreased significantly following preload intake of both milkshakes (all $P < 0.001$). There were no significant differences in terms of delta fullness, hunger, and ratings of prospective consumption of sweet milkshake in comparison to complex carbohydrate milkshake (i.e. differences in fullness, hunger, and ratings of prospective consumption before and after preload consumption between both milkshakes) (all $P > 0.05$).

### Table 8.7 Drinking rates and meal durations of fifty-six adults ($n=56$) for ad libitum consumption of two types of milkshakes containing different amounts of glucose (sweet milkshake) and maltodextrin (complex carbohydrate milkshake).\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Sweet milkshake</th>
<th>Complex carbohydrate milkshake</th>
<th>$P^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinking rate, g/sec</td>
<td>5.8 ± 1.8</td>
<td>3.3 ± 0.2</td>
<td>0.16</td>
</tr>
<tr>
<td>Energy intake rate, kJ/sec</td>
<td>25.7 ± 8.0</td>
<td>15.2 ± 1.0</td>
<td>0.18</td>
</tr>
<tr>
<td>Meal duration, sec</td>
<td>50.2 ± 6.1</td>
<td>49.0 ± 5.4</td>
<td>0.85</td>
</tr>
</tbody>
</table>

\(^1\) Values are adjusted means ± SEMs.

\(^2\) $P$ values representing differences between sweet milkshake and complex carbohydrate milkshake (paired t tests).

$P < 0.05$
8.4.5 Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, and Ad Libitum Intake of Milkshakes

To verify that free sugars in complex carbohydrate solutions were below DTs, if a participant was able to detect glucose in water (DT) at the lowest concentration (0.05 % w/v), potentially that would trigger detection for maltodextrin solution at step 6 (total sugars in maltodextrin: 0.032 % w/v).

There were no significant differences in sweet taste function and oral complex carbohydrate sensitivity (DTs and STs for glucose and maltodextrin) between sex and BMI categories (all $P > 0.05$), therefore, the data are presented together. The frequency distribution of the mean detection thresholds and psychophysical functions of the intensity ratings for both glucose and maltodextrin are shown in Figure 8.5 and Figure 8.6. The DT and mean intensity ratings, standard error of means, and range for both glucose and maltodextrin are presented in Table 8.8. No significant correlations were observed between any measures of sweet taste function and oral complex carbohydrate sensitivity (DTs and STs for glucose and maltodextrin) and BMI (all $P > 0.05$). Significant negative correlations were identified between complex carbohydrate DT and ad libitum consumption of complex carbohydrate milkshakes ($r = -0.38$, $P < 0.01$). However, no significant associations were identified between any measures of sweet taste function and ad libitum consumption of sweet milkshakes (all $P > 0.05$).
Figure 8.5 Frequency distributions of detection thresholds \((n = 56)\) for: a) glucose, b) maltodextrin.
Figure 8.6 Psychophysical curves of the group mean (n = 56) and examples of a participant experiencing high intensity and a participant experiencing low intensity for (a) Glucose (b) Maltodextrin. Included in each graph is an example of the mean psychophysical curve, a participant experiencing high intensity (highest curve), and a participant experiencing low intensity (lowest curve) for glucose and maltodextrin. The y-axis is a numerical measure of intensity from the gLMS. The x-axis is the actual concentration in weight over volume percentage.
Table 8.8 Detection threshold (% w/v) and mean intensity rating (gLMS) for glucose and maltodextrin presented as mean, standard error of mean, and range of fifty-six adults (n = 56).\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Detection Threshold ((n = 56))</th>
<th>Mean Intensity Rating ((n = 56))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>Range</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.9 ± 0.09</td>
<td>0.05-1.8</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>3.3 ± 0.4</td>
<td>0.1-11.2</td>
</tr>
</tbody>
</table>

\(^1\) Mean intensity ratings calculated based on the geometric mean score of the three solution ratings (weak, moderate, and strong)

When stratified into tertile groups (according to the complex carbohydrate and sweetener tested and all taste measures), we observed significant differences in terms of \textit{ad libitum} consumption of complex carbohydrate milkshakes between the participant groups who were more sensitive and less sensitive towards carbohydrate (DT only) (Figure 8.7). Participants who were more sensitive towards complex carbohydrate (maltodextrin DT) consumed significantly more complex carbohydrate milkshake (mean intake (grams) = 148.7g; mean intake (kJ) = 688.9kJ) in comparison to less sensitive participants (mean intake (grams) = 100.4g; mean intake (kJ) = 465.3kJ) (all \(P < 0.01\)). Despite differences in complex carbohydrate milkshake intake (~48% greater energy intake), no significant changes in appetite ratings (\textit{i.e.} increase in fullness ratings, decrease in hunger and prospective consumption) were observed between the more sensitive and less sensitive participants towards complex carbohydrate DT (all \(P > 0.05\)). There was a trend for a similar difference with glucose milkshake (\(P= 0.09\); Figure 8.7). There were no significant differences between more and less sensitive participants or those who experienced low intensity or high intensity to glucose (DT, ST) and maltodextrin (ST) for \textit{ad libitum} consumption of sweet and complex carbohydrate milkshakes (all \(P > 0.05\); Figure 8.7).
Chapter 8 – Study 3(b) The Associations between Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, Liking, and Consumption of Ad Libitum Sweet and Complex Carbohydrate Milkshakes

Figure 8.7 (A-B) Ad libitum milkshake intake mean and standard errors between more sensitive and less sensitive participants or those who experienced high and low intensity ratings (C-D) Ad libitum milkshake intake mean and standard errors between participants with high hedonic ratings and low hedonic ratings for both sweet and complex carbohydrate solutions and prototypical foods. For sweet taste function and sweet hedonic ratings, comparisons were only made for sweet milkshakes, and vice versa for complex carbohydrate. ** P value < 0.01.
8.4.6 Hedonic Ratings for Sweet and Complex Carbohydrate Solutions, Prototypical Foods, Milkshakes, and Ad Libitum Intake of Milkshakes

No significant differences in hedonic ratings for sweet and complex carbohydrate solutions and prototypical foods were identified between sex and BMI groups (all $P > 0.05$). The mean hedonic ratings, standard error of means, and range for both sweet and complex carbohydrate solutions and prototypical foods are presented in Table 8.9. Liking of sweet milkshake was significantly correlated with sweet milkshake intake ($r = 0.35; P < 0.01$). No significant associations were observed between other sweet and complex carbohydrate hedonic measures (solutions, prototypical foods) and ad libitum intake of milkshakes (all $P > 0.05$). When stratified into tertile groups (according to liking ratings towards solutions, prototypical foods, and milkshakes), we observed significant differences in terms of ad libitum consumption of sweet milkshakes between participants with high hedonic ratings and low hedonic ratings for glucose solutions (Figure 8.7). Participants who rated high on the hedonic scale for glucose solutions (high liking) consumed significantly more sweet milkshakes (mean intake (grams) = 199.7g; mean intake (kJ) = 887.6kJ) in comparison to participants with low hedonic ratings (mean (grams) = 111.7g; mean intake (kJ) = 496.8kJ) (all $P < 0.05$). Similarly, significant differences in terms of ad libitum consumption of sweet milkshakes were also identified between participants with high hedonic ratings and low hedonic ratings for the sweet milkshakes (Figure 8.8). Participants who had high hedonic ratings for the sweet milkshake consumed significantly more sweet milkshakes (mean intake (grams) = 238.8g; mean intake (kJ) = 1061.4kJ) in comparison to participants with low hedonic ratings (mean (grams) =
549.5g; mean intake (kJ) = 1061.4kJ) (all $P < 0.05$). There were no significant differences between participants who rated low and high on the hedonic scale according to their liking towards sweet (prototypical foods) and complex carbohydrate hedonic ratings (solutions, prototypical foods, milkshake) for ad libitum consumption of milkshakes (all $P > 0.05$; Figure 8.7 and Figure 8.8).

Table 8.9 Hedonic ratings for sweet and complex carbohydrate solutions and prototypical foods presented as mean, standard error of mean, and range of fifty-six adults ($n = 56$).\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Solutions ($n = 56$)</th>
<th>Prototypical Foods ($n = 56$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>Range</td>
</tr>
<tr>
<td>Sweet</td>
<td>4.8 ± 0.2</td>
<td>2.0-9.6</td>
</tr>
<tr>
<td>Complex carbohydrate</td>
<td>3.2 ± 0.2</td>
<td>0.9-7.8</td>
</tr>
</tbody>
</table>

\(^1\)Hedonic rating for solutions calculated based on the geometric mean score of the three solution ratings (weak, medium, and strong). For hedonic ratings of a range of sweet and complex carbohydrate foods, a geometric mean score of the eight food items was used.
Chapter 8 – Study 3(b) The Associations between Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, Liking, and Consumption of Ad Libitum Sweet and Complex Carbohydrate Milkshakes

*Figure 8.8* Ad libitum milkshake intake mean and standard errors for participants with high hedonic ratings and low hedonic ratings for both sweet and complex carbohydrate milkshakes. For sweet hedonic ratings, comparisons were only made for sweet milkshakes, and *vice versa* for complex carbohydrate. *P* value < 0.05

8.5 Discussion

The present study focused specifically on individual differences in oral sensing of complex carbohydrate and the relationship with liking of complex carbohydrate dominant foods, and satiation in a convenience sample of adults considered non-restrained eaters. To our understanding, the present study is the first to investigate if our ability to detect and perceive complex carbohydrates at a range of concentrations is associated with *ad libitum* intake of energy/foods in the form of liquid. The major finding was that those who were able to detect complex carbohydrate in water at a lower concentration (DT, more sensitive group) consumed 48% more of the complex
carbohydrate milkshake than of those who were less sensitive to complex carbohydrate. Despite differences in intake of complex carbohydrate milkshake, there were no significant changes in appetite ratings (i.e. decrease in hunger and prospective consumption, increase in fullness) between those who were more sensitive and less sensitive to complex carbohydrate (DT). The present study also showed that those who had high hedonic ratings for sweet solutions and milkshake consumed more of the sweet milkshake than those who had lower hedonic ratings. All in, these data suggest a novel role of the oral perceptual system to complex carbohydrates in regards to the overconsumption of energy within a meal.

In the present study, participants who were more sensitive to complex carbohydrate (DT) consumed more of the complex carbohydrate milkshake, thus energy intake, than less sensitive participants. This is in line with our previous studies showing that oral sensitivity to complex carbohydrate is positively associated with habitual energy intake and intake of dietary starch (Chapter 6). In the present study, we found that those who rated higher on the hedonic scale for sweet solutions and sweet milkshakes had greater consumption of the sweet milkshakes. In contrast, despite an increase in consumption between participants who were more sensitive to complex carbohydrate, no significant associations were found between complex carbohydrate liking and ad libitum intake of the complex carbohydrate milkshakes. This suggests a possibility of some sub-conscious mechanism relating to oral sensitivity to complex carbohydrates, but not conscious liking that encourages consumption. These observations have been previously established in rodents and raise the possibility that oral perception from complex carbohydrates is associated with consumption of complex carbohydrate foods (see review by Sclafani (2)). While contentious, the ability to identify complex carbohydrates in foods could serve as a
survival response for humans, especially at times where foods are scarce, as complex carbohydrates represent a major source of energy for body functioning. It could be argued that sweet taste perception or the degree of sweetness in general is not a good proxy for the amount of energy available in a food. (68, 116) Thus, the physiological regulation and functional significance of sensing low amounts of complex carbohydrates could be an advantage, as complex carbohydrates represent a major source of energy for physiological functions. (67, 68) However, since the hunter-gatherer times, the food environment has changed drastically; we now live in an environment with abundance of low-cost, high-energy foods – thought to be one of the major contributors to the global rise in being overweight and obese. (31, 32) Thus, from an evolutionary standpoint, it is possible that oral insensitivity towards complex carbohydrates could function as an evolutionary adaptation response to the environmental changes in food sources. All in, the present finding suggests an important role for oral complex carbohydrate sensitivity in the overconsumption of dietary starch.

The present study showed no significant differences between participants who were more sensitive and less sensitive participants or those who experienced low and high intensity to sweet taste (both DT, ST) and \textit{ad libitum} intake of sweet milkshakes. This is in line with a large body of evidence indicating no significant associations between sweet taste function, BMI, and dietary intake (\textit{Chapter 4}). (158, 189-196, 299) However, in this study we found that the participants who had higher liking ratings for sweet solutions and sweet milkshakes consumed more of the \textit{ad libitum} sweet milkshake than the participants who had lower ratings. It is likely that no significant associations were found between sweetness liking, BMI, and intake of sweet foods in the previous studies, (177, 190, 192, 194, 195, 197-199) as most of
these studies looked at self-reported habitual or usual intake rather than satiation/acute intake in a controlled laboratory environment. Furthermore, it is also possible that no associations were observed between liking and consumption of sweet foods in the previously mentioned studies, as foods high in dietary sugar are most likely accompanied by other taste qualities such as salty, sour, bitter, and fatty tastes. Thus, by matching both sweet and complex carbohydrate milkshakes in energy, serving size, protein, fat, as well as salt and fibre levels, we were able to observe the direct influence of liking of sweetness on intake of a sweet milkshake within a meal. Therefore, foods high in dietary sugar may be one of the many risk factors for overconsumption of energy for individuals with high liking for sweetness due to the sweet taste or flavours present.

The role of sweet taste per se in appetite and food intake regulation remains contentious. The present study and others found that simple (sweet) carbohydrates were less effective in suppressing appetite for a meal than non-sweet foods, (201-204) whereas most studies show no effect of sweet taste on appetite and food intake. (205-209) In this study, both the sweet and complex carbohydrate milkshakes suppressed hunger and prospective consumption and increased fullness after the preload milkshake. However, participants on average drank more (~+32% energy intake) of the ad libitum sweet milkshake in comparison with the non-sweet complex carbohydrate milkshake. It could be argued that the discrepancies between satiation studies could be due to differences in the physical form of the carbohydrates (i.e. liquid, semi-liquid, and solids). For example, it has been suggested that energy in the form of liquid is less satiating in comparison to other forms of energy, (303, 350) and consumption of liquid energy could result in weight gain. (34-36, 374-376) Furthermore, it was observed in a 21-week, randomised crossover study measuring the
Chapter 8 – Study 3(b) The Associations between Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, Liking, and Consumption of Ad Libitum Sweet and Complex Carbohydrate Milkshakes

effects of fruits and vegetables in a solid versus beverage forms on satiation, satiety, and dietary compensation in healthy lean (n=15) and overweight/obese (n=19) adults, that the overweight/obese participants experienced weaker acute satiation and satiety effects of beverage in comparison to solid food forms. (376) For the lean participants, the hunger ratings were not significantly different between the beverage and solid forms. (376) In contradiction with the aforementioned studies, there is also a large body of clinical literature in support for the consumption of sweetened beverages for the purpose of weight loss (see meta-analysis review by Heymsfield et al. (377)). In truth, the diverging results of studies addressing the satiating power of liquids could be due to many factors ranging from behavioural intent, context, availability and cost of sweetened liquids, as well as the mode of use (Drewnowski & Bellisle, 2007). Furthermore, the association between sensitivity or preference and intake is a complex interaction. The current findings could be random observations, but could also be linked with amylase activity.

In contradiction to our hypothesis, this study did not find any association between oral suprathreshold intensity perception for complex carbohydrate and ad libitum consumption of the complex carbohydrate milkshake. However, significant association was observed between detection threshold for complex carbohydrate and ad libitum consumption of the complex carbohydrate milkshake. It has been suggested in previous studies investigating the effects of other taste functions (e.g. salt) and intake that intensity ratings are more appropriate when assessing associations with intake in comparison to threshold measures. (69, 179, 360, 364, 378) In our previous work (Chapter 6), both threshold and suprathreshold intensity ratings (but mostly intensity) were associated with greater habitual energy and starch intakes. A possible explanation for the lack of associations between oral complex carbohydrate sensitivity
and intake of complex carbohydrate using the suprathreshold measure is that the present study examined acute intake of liquid based foods and not habitual intake of complex carbohydrates. Perhaps, it may be the case that detection thresholds for oral complex carbohydrate sensitivity may be more appropriate when assessing acute intake of liquid complex carbohydrates, whereas intensity ratings may be a more precise measure for habitual intake of solid complex carbohydrate foods.

This study needs to be considered alongside limitations, which may have confounded the results. First, the present study measured the intake of only a single food (milkshake). Although laboratory setting research using single foods is the most sensitive approach and provides clear results when quantifying the role of sensory properties on food intake, in reality, however, we normally consume multiple foods in a much less controlled environment as well as foods that consist of a more complex flavour (Drewnowski & Bellisle, 2007). Therefore, it is difficult to extrapolate these findings to everyday life. Second, we did not measure appetite ratings after consumption of the *ad libitum* milkshakes, or appetite ratings and intake of other foods in subsequent hours following the milkshake experiment as this was beyond the scope of the present study. However, it would be interesting for future studies to investigate this further. Third, as we did not include measurements on thirst before and after drinking the preload milkshakes, it is not possible to know if individual’s thirst level interfered with their milkshake intake. Last, but not least, the participants were mainly young female adults within the normal BMI range, thus the present findings may be difficult to generalise to the broader population. As sugar and starch provide a common and appetitive combination in highly consumed foods such as cereals, sweet tasting vegetables, and various baked goods, a next logical step would be to investigate if
Chapter 8 – Study 3(b) The Associations between Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, Liking, and Consumption of Ad Libitum Sweet and Complex Carbohydrate Milkshakes

there are differences in acute consumption of different combinations of sweet and complex carbohydrate foods.

8.6 Conclusions

Participants who were orally more sensitive to complex carbohydrate (DT) consumed 48% more of the carbohydrate milkshake than those who were less sensitive to carbohydrate. However, no relationships were observed between sweet taste function and ad libitum intake of sweet milkshakes. For sweet taste, the present study showed that those who had higher liking ratings for sweet solutions and milkshake consumed significantly more sweet milkshakes in comparison to those who had lower liking ratings. The results also show that participants on average drank more of the ad libitum sweet milkshakes (~+32% energy intake) in comparison with the non-sweet complex carbohydrate milkshake. However, both the sweet and complex carbohydrate milkshakes suppressed hunger and prospective consumption and increased fullness after the preload milkshake. All in, these results provide support regarding the role of the oral cavity (may be taste system) surrounding sensitivity to complex carbohydrate and the consumption of complex carbohydrate foods within a meal. Furthermore, the present findings also provide insights into the relationship between liking of sweet taste and the potential to overconsume sweet foods within a meal.
Chapter Nine: Summary of Major Findings and Conclusions

9.1 Introduction

According to the Landmark Global Burden of Disease report, obesity was emphasised as the key contributor to a number of major health problems worldwide and as a more significant health crisis than starvation and/or malnutrition. (28) Whilst the nature of obesity is multi-factorial, the prevalence of obesity has been associated with excessive dietary energy consumption, in particular greater intakes of sweet foods. (31, 303) The continued increase in the worldwide prevalence of nutrition-related chronic illness such as obesity necessitates a need to better understand the drivers of sweet food intake. (29, 304, 305) It is thought that one mechanism involved in energy regulation is the individual’s ability to detect or sense sweetness in the mouth, and as such, sweet taste function may play an essential role in modulating sweet food acceptance and/or energy intake. (33) As with all taste qualities, there is large individual variation in the capability to perceive sweet taste (sweet taste function). Inter-individual differences or variability in sweet taste function has been previously observed for sucrose, (70, 153-158, 294) and across a range of sweeteners. (159-161) Perhaps, these large individual variations in sweet taste function may explain why there were individual differences in terms of the amount of sugar or sweet foods consumed habitually. (157) However, the role of sweet taste function in promoting consumption of particular nutrients or foods related with weight gain has long been an area of research, but with mixed experimental support. (55-57, 63, 158, 179, 181, 182, 184, 185, 189, 310) Whether or not environmental influences such as an individual’s habitual intake of sweet foods may be influenced by their sweet taste function, or vice
versa remains controversial. For example, some studies found negative associations between sweet taste function and body mass index (BMI) (less sensitivity to sweet taste was correlated with higher BMIs). (185, 310) On the other hand, the majority of studies found no significant associations between sweet taste function and BMI. (158, 189-195, 379) Given that data are inconsistent, it is important to note that discrepancies between studies may vary as a function to the type of psychophysical methodology used, or even using only one measure of sweet taste function (312) as research has shown that no single sweet psychophysical measure reflects sweet taste function in totality. (70) For example, there are three perceptual dimensions of sweet taste function, namely detection threshold (DT), recognition threshold (RT), and suprathreshold intensity perception (ST). (54, 70) It is also probable that the inconsistencies between studies may be due to the type of sweetener investigated, that is, caloric sweeteners and non-nutritive sweeteners (NNS). In the past decades, a range of new NNS (artificial and natural NNS) have been introduced into our diets. A confounding factor in this area is that NNS generally contain only negligible amounts of energy, thereby decoupling sweet taste and energy. It is therefore important to understand the perceptual relationships between caloric and NNS using a range of psychophysical measures within a single group of individuals, and the associations between sweet taste function, body composition, and dietary intake.

Dietary carbohydrates in the form of complex carbohydrates and simple carbohydrates represent two essential sources of energy in our diet. Except for some fruits, complex carbohydrates are more abundant than simple carbohydrates, but it is sugars with their strong palatable sweetness that are the most taste-visible carbohydrates. (2) Compared to simple carbohydrates, complex carbohydrates have long been assumed invisible to taste. (6, 330) One of the many functions of the taste system is to act as a nutrient-toxin detection system; for example, sweet taste indicates
Chapter 9 – Summary of Major Findings and Conclusions

the presence of energy. (66) However, it has been suggested that sweetness in general is not a good proxy for the amount of energy available in a food. (67, 68) Rather, a detection mechanism encouraging consumption of rich complex carbohydrate foods independent from sweet taste may be advantageous to provide quantitative information about food energy content. (67) Indeed, animal studies provide robust evidence indicating that rodents and even some non-human primates are attracted to the taste of complex carbohydrates derived from maltodextrin. (2, 6, 8, 15, 17, 89, 226, 228, 231, 235, 241, 329, 345, 349, 380-382) It has also been reported recently that humans may perceive complex carbohydrates and that the sensitivity to simple carbohydrates (glucose, sucrose) may be qualitatively independent from that to complex carbohydrates (maltodextrin, glucose oligomers). (18, 19) Remarkably, although Lapis et al. (18) observed large individual variances between participants in terms of the α-amylase activity, taste responsiveness to maltodextrin (DE 20, 10, and 5) was not significantly different between participants with high α-amylase activity and those with low α-amylase activity. While the human taste perception of complex carbohydrate (starch) hydrolysis products has been well investigated by Lapis et al. (18, 21), it needs replication and also extension. For example, the psychophysics of oral complex carbohydrate sensitivity and its associations with sweet taste function across multiple sweeteners has not yet been explored completely. It is important to note, that generally, research that includes taste as a variable uses only one measure of taste function or a limited number of concentrations to measure oral sensitivity to complex carbohydrates. Furthermore, it remains to be verified, whether complex carbohydrates have a unique quality that does not overlap with the five basic taste primaries (sweet, sour, salty, bitter, and umami), and also if an individual’s sensitivity to complex carbohydrates remains stable over numerous testing sessions.
Foods high in dietary carbohydrate (simple carbohydrate, complex carbohydrate) have been shown to have a weaker effect on satiation in comparison to other food groups such as dietary protein (257, 258) and result in overconsumption of energy due to their palatability. (259) Furthermore, it has also been reported by de Graaf, Schreurs (259) that sweet carbohydrates were less effective in suppressing appetite for a meal than non-sweet carbohydrates. The latest progress in our knowledge surrounding sweet taste detection mechanisms for caloric sugars and NNS within the mouth, the brain and the gastrointestinal tract, (56, 82, 88-100, 109, 110, 128, 138-145) combined with their fundamental role in regulating appetite and, thus, dietary energy intake, (125, 128, 133, 137, 143, 144, 150) suggest that abnormalities in any or several of these nutrient sensors may be an underlying factor of why some individuals consume more energy. (74, 151, 152) Therefore, it is important to understand the variation between people in terms of their ability to perceive dietary carbohydrates (both simple and complex carbohydrates), and how these may influence consumption of foods (both dietary intake and satiation). Even though largely hypothetical at the present time, understanding the associations between sweet taste function, oral complex carbohydrate sensitivity, dietary intake, and satiation suggest a fascinating model to understand drivers of carbohydrate food intake.
9.2 Discussion of Major Findings

The aim of this thesis was to assess whether individual variations in sweet taste function and oral complex carbohydrate sensitivity may influence body composition and dietary intake (both habitual and acute intake). A secondary aim was to investigate the psychophysics of sweet taste function and oral complex carbohydrate sensitivity across a range of sweet tastants and complex carbohydrates. The data from this thesis also contributes to the novel research area supporting the existence of oral complex carbohydrate sensitivity in humans. Discussions of the key findings from this thesis are as follows:

Study 1(a): Psychophysical Evaluation of Sweetness Function Across Multiple Sweeteners

This study has been published in Chemical Senses (2016): bjw109 as ‘Psychophysical Evaluation of Sweetness Function Across Multiple Sweeteners’

Study 1(a) hypothesised that there will be significant individual variance in sweet taste sensitivities for each measure of sweet taste function. Participants will be able to be classified into more sensitive/experienced high intensity, normal sensitive/experienced moderate intensity, and less sensitive/experienced low intensity groups according to the sweetness of various sweeteners, and sweet taste measures. This study investigated the three main measures of sweet taste function – DT, RT, and ST across a range of caloric and NNS. This study found strong correlations between DT and RT of all caloric sweeteners (sucrose, glucose, fructose, erythritol), and also between DT and RT of the NNS (sucralose, rebaudioside A). However, DT and RT of caloric sweeteners and NNS were weakly correlated suggesting at least some
independence between the two groups at lower concentrations. This may be due to variances in downstream signalling pathways, or even differences in receptor kinetics as a result of binding to different sites of the sweet taste receptor. (77, 111) Conversely, strong correlations were observed between the sweet ST of caloric sweeteners and NNS, supporting commonality of sweet mechanisms throughout the perceptual range. The finding that, for each sweetener, DTs and RTs were correlated with each other, but not with ST ratings suggests added complexity within the sweet taste system. These observations are supported by previous studies investigating the associations between sweet taste function, where DTs for sucrose were found to correlate poorly with sucrose suprathreshold intensity ratings. (70, 78) This suggests that there are distinct perceptual stages for sweet DT, RT, and ST ratings, with each measure of sweet taste characterising a different component of taste function. (70) Therefore, no single measure of taste function is capable of being a definitive marker of sweet taste function. (54, 70) In the present study, sucrose DT and RT were measured using both the ISO standard method of limits and the more intensive ascending forced choice triangle method. Significant correlations were found between sucrose DT and RT using both methods, confirming the ISO standard method of limits as a reliable method for the rapid estimation of DT and RTs for sweet taste (sucrose). Consistent with previous literature, large inter-individual variation in sweet taste function has been observed in the present study. (70, 153-159, 161, 294) The concentration required to reach DT or RT for a sweetener varied approximately 150-fold across the sample population. There was also large individual difference in perceived sweetness intensity; for example, sucrose (13.7% w/v) was rated 8.8 gLMS by 1 participant and 40.5 gLMS by another. Last but not least, the present study supports previously published data on the relationships between taste functions in other taste qualities, demonstrating weak correlations between the DT and the suprathreshold intensity
measures of erythritol, glucose, and fructose ($r = 0.26-0.29$), but not for the other sweeteners. (54, 69, 70, 76, 179, 302)

**Study 1(b): The Associations between Sweet Taste Function, Body Composition, and Dietary Intake in Adults**

*This study has been published in Nutrients, 8(4), 241 as ‘The Association between Sweet Taste Function, Anthropometry, and Dietary Intake in Adults’*

Study 1(b) hypothesised that no measures of sweet taste function (DT, RT, or ST) will be associated with body composition and dietary intake for all sweeteners. This study investigated the associations between the three common measures of sweet taste function, body composition, and dietary intake among adults using multiple sweeteners. Overall, the current findings indicate that DT and RT are not associated with dietary intake and body weight (BMI and waist measurements). The present findings provide support to previous studies where no experimental evidence for a relationship between measures of sweet taste function and body size was observed. (158, 189-195, 379) In contrast, moderate and near significant relationships observed between energy intake and sweetness intensity of two NNS (sucralose, Rebaudioside A), suggests that intensity ratings are more appropriate when assessing sweet taste associations with energy intake in comparison to sweetness DT and RTs. This supports the general school of thought on psychophysical technique comparisons, (69, 116, 312, 317) where taste thresholds have previously been found to have limited utility in predicting experiences in the real world as threshold measures do not depict the dynamic range of sensory function. Thus, the comparison of the ability of an individual to detect and recognise sweetness from a very small amount of sugar/sweet stimuli
Chapter 9 – Summary of Major Findings and Conclusions

may not be as relevant in terms of understanding food behaviour, when most of the sweet and high-energy foods are within the sweetness intensity perception range. (69, 312) As absolute taste threshold measures are time consuming to complete, sweetness perceptions may seem to be a more time efficient method to assess relationships between individual sweet taste function and energy intake. There are numerous possible explanations for the lack of associations between sweetness perception and body composition. For one, it could be argued that perception of tastant solutions in a laboratory setting bears little relevance to actual intake of real food in everyday life. (318, 319) Furthermore, the impact taste perception has on body composition and diet, especially among adults, is poorly understood. (319) For example, sweet liking and aversions are not always direct predictors of intake, and they do not always associate sweetness with liking. (318) Therefore, it has proved difficult to link adult taste perception with body sizes and diet, whether in a laboratory setting or in the real world. (319)

Study 2(a): Evidence Supporting Oral Sensitivity to Complex Carbohydrates Independent of Sweet Taste Sensitivity in Humans

This study is currently review at Plos One Journal as ‘Evidence Supporting Oral Sensitivity to Complex Carbohydrates Independent of Sweet Taste Sensitivity in Humans’

Study 2(a) hypothesised that humans will have DT and ST for complex carbohydrates. Furthermore, there will be significant individual variance in oral complex carbohydrate sensitivity. Participants will be able to be classified into more sensitive/experienced high intensity, normal sensitive/experienced moderate intensity, and less sensitive/experienced low intensity groups according to the oral sensitivities of two complex carbohydrates, including soluble starch, maltodextrin, and a soluble
fibre oligofructose. At present, our data provide evidence that complex carbohydrates (oligosaccharides: maltodextrin, oligofructose) are perceptible and there were no robust correlations observed between the four basic taste primaries (salty, sour, bitter, and umami tastes; both DTs and STs) and DTs and STs of both complex carbohydrates (maltodextrin and oligofructose). For sweet taste, DTs of the complex carbohydrates (maltodextrin, oligofructose) and all of the sweeteners were not correlated. However, there were moderate correlations between the STs of the complex carbohydrates and sweeteners. However, the most challenging prospective confound is with texture/viscosity, especially at higher concentration levels. Although we observed that some participants were able to consistently differentiate complex carbohydrate solutions from water at the lowest concentration levels tested (0.04% w/v), still, the evidence is not conclusive that the DTs and STs reported were not due to additional textural cues. Therefore, while not diminishing the prospect that oral complex carbohydrate sensitivity could be due to textural differences, the present finding suggests that complex carbohydrates are perceptible in the oral cavity and have a distinct oral sensation that does not overlap with any primary taste qualities. These findings are consistent with Lapis et al. (18), where the STs of maltodextrin (DE5 and 10) were not significantly correlated to sodium chloride (salty taste). The present results showed that the DTs of the complex carbohydrates and all of the sweeteners were not correlated, highlighting that mechanisms other than the T1R2-T1R3 are responsible for the detection of complex carbohydrates. In consideration of the concentrations used, it is possible that the participants detected the complex carbohydrates in the maltodextrin samples instead of the free sugars. The current data is consistent with the previous psychophysical studies where participants were found to be able to perceive complex carbohydrates (glucose polymer, glucose oligomers), and that the sensitivity to simple sugar (glucose) was independent of that to complex
carbohydrates. Together, these findings raise the potential existence of an unidentified complex carbohydrate taste receptor in humans that responds to complex carbohydrates independently of that to sweet tastants (at lower concentration levels). (8) Interestingly, strong correlations between the STs of both complex carbohydrates (maltodextrin, oligofructose), but moderate correlations between the STs of complex carbohydrates and sweeteners were observed. One possibility is that a novel receptor might still be involved in the transduction mechanism used to detect complex carbohydrates, but only for the detection range. At the perceptual range, the perception of complex carbohydrates can be partly mediated by the T1R-independent sweet sensing pathways in addition to the putative complex carbohydrate detection receptor. It is also possible that the salivary α-amylase enzymes and free sugars in complex carbohydrates combine to activate the T1R2-T1R3 sweet taste receptor and/or T1R-independent sweet pathway in taste receptor cells thereby showing commonality with sweet taste in the perceptual range. However, at the detection range, maltose/maltotriose has a weak intensity of sweetness and the amount of free sugars in complex carbohydrates may be too low to activate the salivary α-amylase enzymes. Thus, this explanation may potentially explain why we only observed commonality with the sweet taste mechanism for the perceived intensity range, but not at the detection ranges.

Study 2(b): The Associations between Oral Complex Carbohydrate Sensitivity, Body Composition, and Dietary Intake in Adults

This study is currently review at Journal of Nutrition as ‘The Associations between Oral Complex Carbohydrate Sensitivity, Anthropometry, and Dietary Intake in Adults’
Study 2b hypothesised that there will be a positive association between oral complex carbohydrate sensitivity (ST only), body composition, and dietary intake, but no association between oral complex carbohydrate sensitivity (DT), body composition, and dietary intake. To our knowledge, this was the first study to examine if oral complex carbohydrate sensitivity (DTs and STs for both complex carbohydrates) was related to body composition measurements (BMI, waist circumferences) or dietary intake (energy and macronutrient intakes). In the present study, participants who experienced high intensity/were more sensitive to maltodextrin also consumed significantly more total energy per day (both diet diary and FFQ data). It could be argued that the physiological regulation and functional significance of sensing low amounts of complex carbohydrates could be an advantage, as complex carbohydrates represent a major source of energy for physiological functions. In a recent review by Beauchamp (67) on sweet taste, the author noted based on a review paper by Ramirez (68) that sweet taste perception or the degree of sweetness in general is not a good proxy for the amount of energy available in a food. Rather, a detection mechanism encouraging consumption for complex carbohydrate independent from sweet taste may be advantageous to provide quantitative information about the energy (glucose) content for physiological functioning. (67, 68) Considering the small amount of free sugars available in the complex carbohydrates, it could be possible for participants to perceive simple carbohydrates instead of the complex carbohydrates in the samples. However, it is unlikely given that some participants were able to detect complex carbohydrates at low concentration levels, but the same participants were only able to detect simple carbohydrates at higher concentrations (e.g. one participant was able to detect maltodextrin at 0.04% w/v (Glucose: 0.0003% w/v, total sugars in maltodextrin: 0.0011% w/v)). (344) These data provide confidence that we are measuring a phenomena that is not triggered by the small amount of simple carbohydrates in the
samples. These results could be partly explained by animal models. For example, in a study by Sclafani (16) investigating the effects of carbohydrate type (simple carbohydrate versus complex carbohydrate) on body weight and diet in rats, no significant differences in total caloric intake, weight gain, percent body fat, or basal insulin level were observed between groups of rats fed either simple carbohydrate or complex carbohydrate solutions in addition to chow and water for 40 days. However, the complex carbohydrate (maltodextrin) solution group consumed significantly more maltodextrin solution than the simple carbohydrate group over the 40-day period, (16) suggesting that long-term exposure to complex carbohydrates does not reduce subsequent intake of complex carbohydrates. Although differences between rats in terms of their sensitivity towards complex carbohydrates were not investigated, in the context of what we found in the present study, it is possible that the more sensitive participants or those who experienced high intensity consumed more energy and starch, as they could sense complex carbohydrates in foods with lower amounts of complex carbohydrates, in comparison to less sensitive participant or those who experienced low intensity (i.e. some participants in the present study could detect complex carbohydrates as low as 0.04% w/v in water). However, it is also possible that waist circumference influences oral sensitivity to complex carbohydrate rather than the reverse. For example, previous research has suggested the link between high visceral fat depots with distinct endocrine secretion and metabolic profiles, which could possibly influence taste. In the present study, we observed that waist circumference differed between the more sensitive and less sensitive groups of participants and between those who experienced high and low intensity for all complex carbohydrate measures (except oligofructose DT). Oral sensitivity towards complex carbohydrates (DTs and STs) was also negatively associated with energy and starch intakes raises the possibility that food intake, may, in part, be regulated by both oral
sensory and post-digestive nutritive feedback. That is, it is possible that individuals with heightened oral sensitivity responses towards complex carbohydrates may have developed preferences for complex carbohydrate flavours due to post-digestive nutritive cues (conditioned preferences), leading to greater intakes of energy and starch, and thus weight gain. (346-348) Post-digestive modulation of complex carbohydrate intake has previously been established in rat models. For example, Sclafani and Nissenbaum (349) observed that rats rapidly develop preferences for flavours associated with intragastric infusions of maltodextrin. These differences were also observed in long-term tests where rats switched their initial preference for sucrose powder to a preference for bitter-tasting maltodextrin solutions after continued observation over 24-hours, suggesting that these differences were observed due to the post-digestive nutritive feedbacks (i.e. rate of absorption as influenced by diet form).

(2) Taken together, these results reveal a novel association between complex carbohydrate sensing and the consumption of complex carbohydrates. Whether or not oral sensitivity to complex carbohydrate influences waist circumference or vice versa still remains to be investigated.

**Study 3(a): The Associations between Oral Complex Carbohydrate Sensitivity, BMI, Liking, and Consumption of Complex Carbohydrate Based Foods**

**Study 3(a)** hypothesised that participants will have detection threshold and suprathreshold intensity for complex carbohydrates (maltodextrin). There will be individual variance in oral complex carbohydrate sensitivity. Participants will be able to be classified into more sensitive/experienced higher intensity, normal
sensitive/experienced moderate intensity, and less sensitive/experienced lower intensity groups according to the sensitivities of complex carbohydrates (maltodextrin). Furthermore, there will be positive associations between oral suprathreshold intensity perception for complex carbohydrate, BMI, liking, and consumption of complex carbohydrate based foods, but no associations between detection threshold for complex carbohydrate, BMI, liking, and consumption of complex carbohydrate based foods. The present findings replicate those of Lapis et al. (18, 21) and recent observations from our own laboratory, signifying that humans may perceive complex carbohydrates in the oral cavity; and contest the knowledge where complex carbohydrates have been assumed to be invisible to the human palate system. (6, 330-332) Consistent with our previous findings (Study 2(a)), inter-individual variation to oral complex carbohydrate sensitivity varied considerably for this sample, and individuals may be classified as more or less sensitive and as those who experienced higher or lower intensity to complex carbohydrate across both measures (DT and ST). For example, there were large individual differences in participant’s DT for complex carbohydrate (i.e. concentration required to reach DT for this sample population ranged roughly around 120-fold across the sample population). Similarly, for intensity ratings, there were also large individual variances in terms of participant’s perceived complex carbohydrate intensity ratings. Considering the concentrations used, it is possible that the participants detected the complex carbohydrates in the maltodextrin samples instead of the sugars. For example, one participant was able to differentiate the maltodextrin sample from water at 0.2% w/v (Glucose: 0.0018% w/v; total sugars in maltodextrin: 0.0034% w/v) but only able to detect glucose at 1.8% w/v. With the methodologies implied to measure oral complex carbohydrate sensitivity, we are optimistic that the participant’s DT and ST observed for complex carbohydrate were based on their oral sensitivity to complex carbohydrates, and not on other
Chapter 9 – Summary of Major Findings and Conclusions

orosensory clues such as olfaction and visual. However, we could not rule out the possibility that at higher concentration levels some participants may be able to detect differences between samples due to texture/viscosity. Although inter-individual variation to oral complex carbohydrate sensitivity is large, and there were participants who were able to detect complex carbohydrate in water even at the lowest concentrations tested (0.1% \text{w/v}), yet, the evidence is not conclusive that the DT and ST reported for maltodextrin were not due to additional textural cues. The current findings also indicate that oral complex carbohydrate sensitivity (DT and ST) are not associated with the frequency of consumption of complex carbohydrate based foods measured. A possible explanation for the lack of associations between oral complex carbohydrate sensitivity and the consumption of complex carbohydrate based foods is that the present study examined individual foods only whereas the other study (Study 2b) examined percentage energy from starch and overall energy intake and not individual foods. Perhaps, it may be the case that the frequency of intake of carbohydrate based foods is actually unlikely to differ between people who are more or less sensitive or of those who experienced higher intensity or lower intensity, but rather, what may be going on is that people who are more sensitive or experienced higher intensity may consume greater quantities of complex carbohydrate based foods when they are consumed (which we did not assess in this study). The observation that BMI measurements do not differ between measures of oral complex carbohydrate sensitivity (DT and ST) suggests that BMI is not regulated by oral sensitivity towards complex carbohydrate. Our recent research provides support for this proposition as we observed significant differences in oral complex carbohydrate sensitivity (maltodextrin, oligofructose) amongst participants with smaller and larger waist circumference measurements (Chapter 2b). That is, more sensitive participants or those who experienced higher intensity towards both complex carbohydrates
(maltodextrin, oligofructose) had on average more than 10 cm differences in terms of waist circumference measurements in comparison to less sensitive participants or those who experienced lower intensity. Finally, the present study found no significant differences in terms of sweet liking (both solutions and prototypical foods), body weight, and frequency of consumption of sweet foods. Previous data investigating the link between sweetness liking, BMI, and intake of sweet foods is conflicting, with most data failing to find any significant associations. (177, 190, 192, 194, 197-199, 309) Similarly, at present, no significant differences in terms of complex carbohydrate liking (solutions and prototypical foods), BMI, and frequency of consumption of complex carbohydrate based foods were found.
Study 3(b): The Associations between Sweet Taste Function, Oral Complex Carbohydrate Sensitivity, Liking, and Consumption of *Ad Libitum* Sweet and Complex Carbohydrate Milkshakes

Study 3(b) hypothesised that there will be positive correlations between oral suprathreshold intensity perception for complex carbohydrate and *ad libitum* consumption of complex carbohydrate based foods, but no correlations between detection threshold for complex carbohydrate and *ad libitum* consumption of complex carbohydrate based foods. Furthermore, there will be positive correlations between liking towards complex carbohydrates and *ad libitum* consumption of complex carbohydrate based foods. The present study focused specifically on individual differences in oral sensing of complex carbohydrate and the relationship with liking of complex carbohydrate dominant foods, and satiation in a convenience sample of adults considered non-restrained eaters. To our understanding, the present study is the first to investigate if our ability to detect and perceive complex carbohydrates at a range of concentrations is associated with *ad libitum* intake of energy/foods in the form of liquid. The major finding was that those who were able to detect complex carbohydrate in water at a lower concentration (DT, more sensitive group) consumed 48% more of the complex carbohydrate milkshake than of those who were less sensitive to complex carbohydrate. In the present study, we found that those who rated higher on the hedonic scale for sweet solutions and sweet milkshakes had greater consumption of the sweet milkshakes. In contrast, despite an increase in consumption between participants who were more sensitive to complex carbohydrate, no significant associations were found between complex carbohydrate liking and *ad libitum* intake of the complex carbohydrate milkshakes. This suggests a possibility of some sub-conscious mechanism relating to oral sensitivity to complex carbohydrates, but not conscious
liking that encourages consumption. All in, the present finding suggests an important role for oral complex carbohydrate sensitivity in the overconsumption of dietary starch.

9.3 Conclusions

Major conclusions from this thesis are as follows:

- The present thesis highlights the complexity of human sweetness perception: no single measurement of sweet taste function was able to characterise sensitivity, and no one sweet compound was representative of other sweet compounds. The findings are consistent with the proposition of one primary sweet taste receptor for both NNS and caloric sweeteners, with different domains in the receptor.

- Although all measures of sweet taste function differed between individuals for all sweeteners, oral sweet taste sensitivity does not appear to have any robust influence on body composition measurements and dietary intake. Sweetness intensity from two NNS was associated with energy intake indicating that sweetness intensity measures might be more appropriate when assessing associations with total energy intake. There were no associations found between body composition measurements and sweet taste function across a range of sweeteners. Supporting this, there were no differences in sweet taste function between lean and overweight/obese participants.

- The data highlight that complex carbohydrates (maltodextrin, oligofructose) are perceptible in the oral cavity and have a distinct oral sensation that does not overlap with any primary taste qualities. Additionally, our data indicate that oral sensitivity to complex carbohydrate is not related to a range of sweeteners
at low concentration levels (DTs). The findings are consistent with the proposition of an independent mechanism for complex carbohydrates, but only for lower concentration levels. At the perceptual range, it is possible that the perception of complex carbohydrates may be partly mediated by the T1R-independent sweet sensing pathways in addition to the putative complex carbohydrate detection receptor. Another possibility is that the salivary $\alpha$-amylase enzymes and free sugars in complex carbohydrates combine to activate the T1R2-T1R3 sweet taste receptor and/or T1R-independent sweet pathway in taste receptor cells thereby exhibiting the commonality with sweet taste in the perceptual range.

- Participants who were orally more sensitive or those who experienced high intensity for complex carbohydrates consumed more energy and starchy foods per day, and had bigger waist circumference measurements in comparison to participants who were orally less sensitive or experienced low intensity. These results suggest that oral complex carbohydrate sensitivity could play a role in energy and starch intake regulation, which may determine body size measurements. However, it is also possible that waist circumference could influence oral sensitivity to complex carbohydrate rather than the reverse. Taken together, these results reveal a novel association between oral complex carbohydrate sensing and the consumption of complex carbohydrates. Whether or not oral sensitivity to complex carbohydrate influences waist circumference or vice versa still remains to be investigated.

- There are large inter-individual variations in oral complex carbohydrate sensitivity measures (DT and RT), and participants could be grouped into more sensitive and less sensitive or those who experienced higher and lower intensity groups for complex carbohydrate (maltodextrin) in a large convenience sample.
Chapter 9 – Summary of Major Findings and Conclusions

of adults. No significant differences were found between oral complex carbohydrate sensitivity, BMI, and frequency of consumption of the complex carbohydrate based foods measured. Similarly, no differences were observed between liking of complex carbohydrates, BMI, and food intake. All in, these results provide strong support for the proposition that complex carbohydrates are not invisible to the human palate, and can be sensed in the oral cavity even at low concentration levels in a large sample group.

- One of the major findings from this thesis was that those who were able to detect complex carbohydrate in water at a lower concentration (DT, more sensitive group) consumed 48% more of the complex carbohydrate milkshake than those who were less sensitive to complex carbohydrate. Despite differences in intake of complex carbohydrate milkshake, there were no significant changes in appetite ratings (i.e. decrease in hunger and prospective consumption, increase in fullness) between those who were more sensitive and less sensitive to complex carbohydrate (DT). The present study also showed that those who had higher hedonic ratings for sweet solutions and milkshake consumed more of the sweet milkshake than those who had lower hedonic ratings. All in, these data suggest a novel role of the oral perceptual system to complex carbohydrates in regards to the overconsumption of energy within a meal.
Data investigating the link between sweet taste function, dietary intake, and BMI has been controversial. Discrepancies between studies may be attributed to the type of psychophysical methodology used, or the sweeteners used to measure sweet taste. Collecting a range of psychophysical measures across a range of sweeteners (both caloric and NNS) within a single group of individuals allows direct comparison that cannot be made across prior studies. In this way, the studies conducted in this thesis have added to the growing body of literature on the psychophysics of sweet taste across a range of sweeteners, as well as the associations between sweet taste function, body composition, and dietary intake. Additionally, as part of this thesis, the psychophysics of complex carbohydrates and its associations with the six common sweeteners were also investigated. As complex carbohydrates such as maltodextrin contains small amounts of glucose, maltose, and maltotriose, the next logical step would be to investigate the perceptual relationships between maltodextrin, glucose, maltose, and maltotriose. Furthermore, although many participants were able to consistently differentiate complex carbohydrate solutions from water even at low concentration levels (i.e. 0.04% w/v), more evidence from tribology studies is required to ensure that the DTs and STs reported were not due to textural cues.

The area of oral complex carbohydrate sensitivity is a novel research topic and there are many gaps in the knowledge base. In the present study, being more sensitive/experiencing high intensity to complex carbohydrates were associated with greater energy and starch intakes (both habitual and acute intake) and bigger waist measurements. However, the role of diet and appetite regulation and genetics remains to be investigated. Therefore, dietary intervention studies that involve assessing an individual’s oral complex carbohydrate sensitivity over time during a high or low
complex carbohydrate diet, along with tongue papillae biopsy to investigate if there is any associated receptor(s) in the human oral cavity would be warranted. Whilst unconfirmed at this stage, it may be long-term complex carbohydrate intake that modulates oral complex carbohydrate sensitivity. As the identity of the taste receptor(s) for complex carbohydrate remains to be identified, studies to find potential receptor(s) is required. Furthermore, it would also be important to determine a concentration that can be used to reliably classify people into more sensitive or less sensitive groups to both simple and complex carbohydrates.

One of the main conclusions from this thesis was that those who were more sensitive to oral complex carbohydrate (maltodextrin) consumed significantly more of the complex carbohydrate milkshake than those who were less sensitive to complex carbohydrate. However, it remains to be investigated if this excess energy intake would be compensated in subsequent meals. Therefore, future research investigating the satiation and satiety effects over a longer period of time after drinking a complex carbohydrate preload will be needed to gain full understanding of this topic. As sugar and starch provide a common and appetitive combination in highly consumed foods such as cereals, sweet tasting vegetables, and various baked goods, a next logical step would be to investigate if there are differences in acute consumption of different combinations of sweet and complex carbohydrate foods. Furthermore, due to the observation that caloric sweeteners and NNS may access at least partially independent mechanisms, future research should investigate if there are differences in acute consumption of sweet foods (caloric versus NNS).
Chapter Ten: References


5. Richardson B. Sugar: refined power in a global regime: Springer; 2009.


20. Lim J, Lapis T, Son H, Rhyu M, Lim J, editors. Detection of Glucose Oligomers by Humans is Not through the Human Sweet Taste Receptor. CHEMICAL SENSES; 2016: OXFORD UNIV PRESS GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND.


122. Tordoff MG, Ellis HT. Taste dysfunction in BTBR mice due to a mutation of Itpr3, the inositol triphosphate receptor 3 gene. Physiological genomics. 2013.


165. Eny KM, Wolever TM, Corey PN, El-Sohemy A. Genetic variation in TAS1R2 (Ile191Val) is associated with consumption of sugars in overweight and obese individuals in 2 distinct populations. The American journal of clinical nutrition. 2010:ajcn. 29836.


324. Donaldson LF, Bennett L, Baic S, Melichar JK. Taste and weight: is there a link? The American journal of clinical nutrition. 2009;90(3):800S-3S.


“In every job that must be done, there is an element of fun”

– Mary Poppins
Appendix A
Screening Form (Study 1 and Study 2)
Screening Questionnaire
To be filled in by Researcher Only

1. Are you older than 18 years of age: Y / N

2. Date of birth (Date/Month/Year): __________________________

3. Are you currently pregnant or lactating (for females only): Y / N

4. Are you a smoker? Y / N

5. Are you currently taking any prescription medication that may interfere with your ability to taste? (*If no, move to question 6) Y / N

6. Please list medications that you are currently taking in the space provided:

   __________________________________________________________
   __________________________________________________________
   __________________________________________________________
   __________________________________________________________

7. Do you have any food allergies? (*If no, ignore question 7) Y / N

8. List your allergies in the space provided.

   __________________________________________________________
   __________________________________________________________
   __________________________________________________________
   __________________________________________________________

***
Screening Questionnaire
To be filled in by Researcher Only

1. Are you older than 18 years of age: Y / N

2. Date of birth (Date/Month/Year): __________________________

3. Are you currently pregnant or lactating (for females only): Y / N

4. Are you a smoker? Y / N

5. Are you currently taking any prescription medication that may interfere with your ability to taste? (*If no, move to question 6) Y / N

6. Please list medications that you are currently taking in the space provided:
   *NO MEDICATIONS LISTED KNOWN TO INTERFERE WITH PARTICIPANT’S ABILITY TO TASTE

7. Do you have any food allergies? (*If no, ignore question 7) Y / N

8. List your allergies in the space provided.
   *NO HISTORY OF FOOD ALLERGIES TOWARDS INGREDIENTS USED IN THE STUDY
Appendix B
Recruitment Flyer Study 1
Are artificial sweeteners good for weight loss?

WE NEED YOUR HELP!!!

We are looking for volunteers who would like to participate in a study investigating the associations between sweet and carbohydrate taste function (if you are more or less sensitive towards different kinds of sugar/sweeteners) with food consumption and Body Mass Index.

Are you:

- Over the age of 18?
- A non-smoker?
- Able to attend sessions at Deakin University in Burwood where you will taste primarily sweet taste solutions?

If you are interested in learning more about the study, please contact Julia at 03 xxxxxxx or xxxx@deakin.edu.au
Appendix C
Recruitment Flyer Study 2
We are looking for **non-smoking volunteers over 18 years of age** to participate in a study that aims to investigate if we can taste different carbohydrates.

Participation involves:
- Attending sessions at the Burwood Campus (12.14) to taste solutions with various carbohydrate contents
- Completing questionnaires on dietary habits

**All participants will receive a $100 Wish Gift Card**

If you would like to participate, or would like further information, please contact Julia Low on xxxxxxxx or xxxx@deakin.edu.au
Appendix D
Demographic Questionnaire- Online Survey and Compusense Script (Study 3)
Welcome to the HSN313 Laboratory Questionnaires

Thank you for taking your time to fill in the questionnaires.

1. Student ID number

2. Name (First Name, Last Name)

3. HSN313 laboratory class time (e.g. Monday 8am, Monday 11am, Monday 2pm etc.)
4. Gender
   - Male
   - Female

5. If you are a female, are you currently pregnant or lactating?
   - N/A
   - Yes
   - No

6. Year of birth (e.g. 1994, 1995 etc)

7. Nationality

8. Are you a smoker within the last 2 years?
   - Yes
   - No
Student ID: ______________________________

Gender: ____________________________

Which year were you born (e.g. 1994): ____________________________

Are you currently pregnant or lactating?
  - N/A
  - Yes,
  - No

Are you currently suffering from a cold/flu?
  - Yes
  - No

Are you currently consuming any medications that may interfere with your ability to taste?
  - Yes
  - No

Are you a smoker in the past 2 years?
  - Yes
  - No

Do you have any food allergies?
  - Yes
  - No

List your allergies in the space provided.
Appendix E
Three Alternate Forced Choice Form (Study 1 and Study 2)
Participant ID: ____________________

<table>
<thead>
<tr>
<th>Concentration</th>
<th>1&lt;sup&gt;st&lt;/sup&gt;</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt;</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix F
Detection Threshold Questionnaire – Compusense Script
(Study 3)
Questionnaires text Compusense (Monday 9am)
Laboratory 1

Tray 1

For the next 8 samples, please follow the instructions below:

1. Please put on your nose clip.
2. Starting from the sample on the left of your tray, rinse the entire 15 mL sample through your mouth.
3. Hold the sample in your mouth and swirl it around for 5 seconds.
4. **Do not swallow the sample.**
5. Rate the perception of the taste.
6. Please ensure the three-digit code on the sample you taste is identical to that on answer sheet.
7. Before moving onto the next sample, please rinse your mouth with water.

***

a) Rate the perception of the taste of sample 431
   - The solution tastes like water
   - The solution tastes like something other than water, but I am not quite sure what taste it is

b) Rate the perception of the taste of sample 115
   - The solution tastes like water
   - The solution tastes like something other than water, but I am not quite sure what taste it is

c) Rate the perception of the taste of sample 610
   - The solution tastes like water
   - The solution tastes like something other than water, but I am not quite sure what taste it is

d) Rate the perception of the taste of sample 770
   - The solution tastes like water
   - The solution tastes like something other than water, but I am not quite sure what taste it is

e) Rate the perception of the taste of sample 129
   - The solution tastes like water
   - The solution tastes like something other than water, but I am not quite sure what taste it is

f) Rate the perception of the taste of sample 263
   - The solution tastes like water
   - The solution tastes like something other than water, but I am not quite sure what taste it is

g) Rate the perception of the taste of sample 350
   - The solution tastes like water
   - The solution tastes like something other than water, but I am not quite sure what taste it is
h) Rate the perception of the taste of sample 487
   ▪ The solution tastes like water
   ▪ The solution tastes like something other than water, but I am not quite sure what taste it is

Tray 2

For the next 8 samples, please follow the instructions below:

1. Please put on your nose clip.
2. Starting from the sample on the left of your tray, rinse the entire 15 mL sample through your mouth.
3. Hold the sample in your mouth and swirl it around for 5 seconds.
4. **Do not swallow the sample.**
5. Rate the perception of the taste.
6. Please ensure the three-digit code on the sample you taste is identical to that on the computer screen.
7. Before moving onto the next sample, please rinse your mouth with water.

***

a) Rate the perception of the taste of sample 521
   ▪ The solution tastes like water
   ▪ The solution tastes like something other than water, but I am not quite sure what taste it is

b) Rate the perception of the taste of sample 424
   ▪ The solution tastes like water
   ▪ The solution tastes like something other than water, but I am not quite sure what taste it is

c) Rate the perception of the taste of sample 847
   ▪ The solution tastes like water
   ▪ The solution tastes like something other than water, but I am not quite sure what taste it is

d) Rate the perception of the taste of sample 948
   ▪ The solution tastes like water
   ▪ The solution tastes like something other than water, but I am not quite sure what taste it is

e) Rate the perception of the taste of sample 635
   ▪ The solution tastes like water
   ▪ The solution tastes like something other than water, but I am not quite sure what taste it is

f) Rate the perception of the taste of sample 187
   ▪ The solution tastes like water
   ▪ The solution tastes like something other than water, but I am not quite sure what taste it is
g) Rate the perception of the taste of sample 195
   ▪ The solution tastes like water
   ▪ The solution tastes like something other than water, but I am not quite sure what taste it is

h) Rate the perception of the taste of sample 490
   ▪ The solution tastes like water
   ▪ The solution tastes like something other than water, but I am not quite sure what taste it is
Appendix G
General Labeled Magnitude Scale (gLMS)
Instructions for GLMS training (FOR RESEARCHER ONLY)

During the training, you will be asked to rate the intensity of the taste solution by indicating using a pen/mouse where on the scale each sensation lies.

As you can see from the scale on the paper given to you/screen in front of you (refer to scale), the scale is partitioned by verbal descriptors of intensity that we commonly use in everyday life. Such as barely detectable, weak, moderate, strong, very strong and strongest imaginable.

As you experience a sensation, you should first determine which descriptor most appropriately describes the intensity of the sensation, then fine tune your ratings by moving your pen/mouse along the line of the scale to the proper location between the descriptors.

Thus, if you consider a sensation is best described as moderate, but that is towards the strong end of moderate, you should draw your line above moderate, but to the appropriate distance from strong (it can be anywhere you think it will fall under). Conversely, if the sensation is on the weak side of moderate, draw the lines to the appropriate location between moderate and weak.

***

In making your judgements of intensity on the scale, you should rate the stimuli relative to other sensations of all kinds that you have experienced in daily life. For example, when you are rating a very strong taste solution, you will have to rate the intensity of the taste in comparison to all other intensity sensations such as hot and cold or intensity which involves pain such as biting your tongue, child birth and etc.

Therefore, this means that what might be considered as a very strong solution, should not be rated as strong on the gLMS because the intensity of the taste may only be moderate or weak compared to all other types of sensation, especially those that involves pain.

Thus, in rating taste sensation, the strongest taste sensation should never be rated as strong on the gLMS, as taste is generally less intense than any sort of pain or stings.

It is also important to emphasise that the top of the scale is the “strongest imaginable” sensation, which represents the most intense and therefore most painful sensation that you can ever imagine experiencing.
To acquaint you with the use of the scale, I would like you to place your pen/mouse on the scale that best describes the intensity of the following remembered or imagined taste sensation:

*Do not tell them the answers, just the intensity range*

1. The warmth of the luke warm water  5  
2. Coolness of an ice-cold beverage  10  
3. The pain from biting your tongue  35  
4. Bitterness of celery  0  
5. Sweetness of cotton candy  20  
6. Soursness of biting into a lemon  25  
7. Burning sensation of a very hot chilli pepper  35
Appendix I
Weight Bottles and Form - gLMS Standardisation
Aluminium Wrapped Weight Bottles

A = 53g, B = 251g, C = 499g, D = 724g, E = 897g, and F = 1127g
Instructions
You will be given a series of containers. Your task is to rate the weight of each container using the gLMS.

If you are right handed, place your left hand out from your body, palm up. The researcher will place the container on your palm and you will rate the weight of the bottle using the scale provided. If you are left handed, place your right hand away from your body, palm up.

Use the same scale for the first six weights, then the second scale for the following six weights. Beside each rating, place a number from 1-6 indicating the order in which the weight was given and rating made e.g., place 1 beside the first rating you make, 2 beside the second rating etc…

Please ask questions if you are unsure about your role during this task.

Thank you for your participation

ID#_______________________
Appendix J
Determination of Common Sugars in Foods by HPLC
(Methods by the Australian Government National Measurement Institute)
## Determination of Common Sugars in Foods by HPLC

<table>
<thead>
<tr>
<th>Analysis Description</th>
<th>Determination of Common Sugars (glucose, fructose, sucrose, maltose &amp; lactose) in Foods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matrix / Matrices</td>
<td>Foods</td>
</tr>
<tr>
<td>Reference Method(s)</td>
<td>AOAC 18th Ed. 31.138-31.142</td>
</tr>
</tbody>
</table>
| Limit of Reporting (LOR) | 0.2 g/100g with refractive index detector.  
0.05 g/100g with ELSD detector. |
| NATA Accredited      | Yes                                                                                      |
| Preparation & procedure | Preparation:  
Sample is homogenised and a sub sample is accurately weighed.  
Sugars are extracted with 25 ml water at 60°C for 30 minutes.  
The extract is clarified with 25 ml acetonitrile and filtered through a 0.45um filter into a 2ml vial, suitable for HPLC.  

Determination for common sugars:  
Filtered solution is analysed by HPLC using amino column with an acetonitrile/water mobile phase containing salt and refractive index detection. Quantitation is made against a standard solution containing known amounts of fructose, glucose, sucrose, maltose and lactose.  

Determination for low level sugars:  
Filtered solution is analysed by HPLC using carbohydrate ES column with an acetonitrile/water mobile phase and evaporative light scattering detector (ELSD). Quantitation is made against a standard solution containing known amounts of fructose, glucose, sucrose, maltose and lactose.  

Calculation:  
Result calculation is performed by HPLC software and a report generated. |
| Comments, limitations or known interferences | Sorbitol, galactose and other sugar alcohols may interfere with glucose or other sugars. When this occurs the glucose is determined using different mobile phase or separately using a Bio-Rad HPX column.  
The method uncertainty is relatively high at levels approaching the Limit of Reporting (0.2g/100g). |
| Equipment used | Flasks and glassware  
Balance  
Blender  
HPLC with RI or ELSD Detection and appropriate column(s)  
Software to perform integration and calculation of results |
| QA Protocols per batch | 1 duplicate each batch (up to 15 samples usually)  
A standard is run every 5 samples  
A control reference is run each batch  
A recovery test in every batch |
| Mass of Sample required | 15 g per sample, however more sample would be required for QA. |
REPORT OF ANALYSIS

Client: DEAKIN UNIVERSITY
Job No.: DEAK02/141211
Quote No.: QT-02039
Order No.: 
Date Sampled: 
Date Received: 11-DEC-2014
Sampled By: CLIENT

Attention: JULIA LOW
Project Name: 
Your Client Services Manager: Tim Stobius
Phone: (03) 9644 4849

<table>
<thead>
<tr>
<th>Lab Reg No.</th>
<th>Sample Ref</th>
<th>Sample Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>V14/027932</td>
<td>1</td>
<td>Maltodextrin</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lab Reg No.</th>
<th>Sample Reference</th>
<th>V14/027932</th>
<th>Units</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Proximates
  - Fructose: g/100g < 0.2
  - Glucose: g/100g 0.8
  - Sucrose: g/100g < 0.2
  - Maltose: g/100g 0.9
  - Lactose: g/100g < 0.2
  - Total Sugars: g/100g 2.8
  - Maltotriose: g/100g 1.1
  - Galactose: g/100g < 0.2

Signed:

Norbert Strobel, Analyst
Food Composition - Vic

19-DEC-2014

Results relate only to the sample(s) tested.
This Report shall not be reproduced except in full.
REPORT OF ANALYSIS

Client: DEAKIN UNIVERSITY

221 BURWOOD HIGHWAY
BURWOOD VIC 3125

Client: Job No.: DEAK003/101118
Quote No.: QT-03094
Order No.: 
Date Sampled: 
Date Received: 18-NOV-2018
Sampled By: 

Attention: JULIA LOW
Project Name: 
Your Client Services Manager: Tim Stobaus
Phone: (03) 9544 4849

<table>
<thead>
<tr>
<th>Lab Reg No.</th>
<th>Sample Ref</th>
<th>Sample Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>V16/030853</td>
<td>1</td>
<td>Multidextrin DE5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lab Reg No.</th>
<th>Sample Reference</th>
<th>Units</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>V16/030853</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Proximates

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Signed: 

Lao Dinh, Analyst
Food Composition - Vic

1-DEC-2016

Results relate only to the sample(s) tested.
This Report shall not be reproduced except in full.
Appendix K
Examples of Five Prototypical Taste Detection and Recognition Threshold Questionnaire – Compusense Script
(Study 1 and Study 2)
Tray 1 (EXAMPLE OF ONE PROTOTYPICAL TASTE QUALITY FOR ONE PARTICIPANT)

For the next 8 samples, please follow the instructions below:

1. Please put on your nose clip.
2. Starting from the sample on the left of your tray, rinse the entire 15 mL sample through your mouth.
3. Hold the sample in your mouth and swirl it around for 5 seconds.
4. **Do not swallow the sample.**
5. Rate the perception of the taste.
6. Please ensure the three-digit code on the sample you taste is identical to that on answer sheet.
7. Before moving onto the next sample, please rinse your mouth with water.

***

a) Rate the perception of the taste of sample 521
   - The solution tastes like water
   - The solution tastes like something other than water, but I am not quite sure what taste it is
   - Sweet
   - Umami (savoury)
   - Salty
   - Bitter
   - Sour

b) Rate the perception of the taste of sample 424
   - The solution tastes like water
   - The solution tastes like something other than water, but I am not quite sure what taste it is
   - Sweet
   - Umami (savoury)
   - Salty
   - Bitter
   - Sour

c) Rate the perception of the taste of sample 847
   - The solution tastes like water
   - The solution tastes like something other than water, but I am not quite sure what taste it is
   - Sweet
   - Umami (savoury)
   - Salty
   - Bitter
   - Sour

d) Rate the perception of the taste of sample 948
   - The solution tastes like water
   - The solution tastes like something other than water, but I am not quite sure what taste it is
   - Sweet
   - Umami (savoury)
e) Rate the perception of the taste of sample 635
   ▪ The solution tastes like water
   ▪ The solution tastes like something other than water, but I am not quite sure what taste it is
   ▪ Sweet
   ▪ Umami (savoury)
   ▪ Salty
   ▪ Bitter
   ▪ Sour

f) Rate the perception of the taste of sample 187
   ▪ The solution tastes like water
   ▪ The solution tastes like something other than water, but I am not quite sure what taste it is
   ▪ Sweet
   ▪ Umami (savoury)
   ▪ Salty
   ▪ Bitter
   ▪ Sour

g) Rate the perception of the taste of sample 195
   ▪ The solution tastes like water
   ▪ The solution tastes like something other than water, but I am not quite sure what taste it is
   ▪ Sweet
   ▪ Umami (savoury)
   ▪ Salty
   ▪ Bitter
   ▪ Sour

h) Rate the perception of the taste of sample 490
   ▪ The solution tastes like water
   ▪ The solution tastes like something other than water, but I am not quite sure what taste it is
   ▪ Sweet
   ▪ Umami (savoury)
   ▪ Salty
   ▪ Bitter
   ▪ Sour
Appendix L
Pictures of Prototypical Sweet and Complex Carbohydrate Based Foods Used to Measure Hedonic Ratings (Study 3)
<table>
<thead>
<tr>
<th>Sweet and Complex Carbohydrate Based Foods</th>
<th>Pictures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney Beans, Coles Red Kidney Beans, 400g canned</td>
<td><img src="image1" alt="Kidney Beans" /></td>
</tr>
<tr>
<td>Pasta, Coles, Pasta Elbows, 500g</td>
<td><img src="image2" alt="Pasta Elbows" /></td>
</tr>
<tr>
<td>Rice, Sunrice, Long Grain White Rice Cup, 2 pack, 250g</td>
<td><img src="image3" alt="Rice" /></td>
</tr>
</tbody>
</table>
White Bread, Coles Smart Buy, 650g

Weet-Bix Sanitarium 1.4kg Value Pack

Pretzel, Parkers, Baked Mini Pretzels, 225g
Rice Cake, Sunrice, Thin Original Gluten Free Cakes, 150g

Rice Cracker, Sakata, Plain, 100g

Diced Tomatoes, Canned, Coles Smart Buy, 400g
Apples, Dried, Angas Park, 200g

Jelly, Aeroplane, Ready To Eat Blackcurrant Jelly, 120g

Cottee’s Squeezy Chocolate Topping, 615g
Strawberry Jam/Conserv e IXL, 250g

Honey, Coles, Squeeze Honey

Dried Raisins, Coles, 375g
Fairy Floss/
Cotton
Candy
(USA)
Appendix M
9-point Hedonic Scale – Questionnaire and Compusense Script for Solutions and Prototypical Foods (Study 3)
**HSN313 Week 1** *(EXAMPLE QUESTIONNAIRE FOR PROTOTYPICAL FOODS)*

Student ID: ________________________________

Please rate how much do you like sample **552**

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like or dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

Please rate how much do you like sample **429**

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like or dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

Please rate how much do you like sample **239**

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like or dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>
Please rate how much do you like sample **698**

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like or dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like much</th>
<th>Like extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please rate how much do you like sample **184**

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like or dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like much</th>
<th>Like extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please rate how much do you like sample **729**

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like or dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like much</th>
<th>Like extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Please rate how much do you like sample 284

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like or dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>

Please rate how much do you like sample 715

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like or dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>
Questionnaire text Compusense (EXAMPLE COMPUSENSE SCRIPT FOR PROTOTYPICAL SOLUTIONS)

Student ID: ***

Tray 1

For the next 4 samples please follow the instructions below:

1. Starting from the sample on the left of your tray, please taste the entire 15 mL sample. Please swallow the sample.

2. Rate your liking of the sample on the scale provided. For each sample, please clearly choose a response from the scale that best represents your liking of that sample.

3. Please ensure the three-digit code on the sample you taste is identical to that on the computer screen.

4. Before moving onto the next sample, please rinse your mouth with water.

***

1. Please rate your liking of sample 238 on the scale provided.

2. Please rate your liking of sample 156 on the scale provided.

3. Please rate your liking of sample 913 on the scale provided.

4. Please rate your liking of sample 258 on the scale provided.

***
Tray 2

For the next 4 samples please follow the instructions below:

5. Starting from the sample on the left of your tray, please taste the entire 15 mL sample. Please swallow the sample.

6. Rate your liking of the sample on the scale provided. For each sample, please clearly choose a response from the scale that best represents your liking of that sample.

7. Please ensure the three-digit code on the sample you taste is identical to that on the computer screen.

8. Before moving onto the next sample, please rinse your mouth with water.

***

5. Please rate your liking of sample 647 on the scale provided.

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like or dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6. Please rate your liking of sample 256 on the scale provided.

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like or dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

7. Please rate your liking of sample 873 on the scale provided.

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like or dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8. Please rate your liking of sample 975 on the scale provided.

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like or dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

***
Appendix N
Likes Dislikes Questionnaire – Example of Printed Version
(Study 3)
Participant ID number:

Background

This questionnaire is designed to assess your like or dislike of a number of food/beverage items and common experiences.

Confidentiality

All the information provided in this questionnaire will be treated in the strictest confidence.

How to fill in the questionnaire

You will rate your like or dislike of a number of food/beverage items and common experiences on a 9-point scale with the middle of the scale being neutral, or neither like nor dislike. There are some categories of foods, for example Cake, which could be any number of varieties such as Banana, Chocolate, Carrot etc. You should rate your liking of cake in general, not necessarily the best cake you have ever eaten.

For each item, please clearly mark the check box that best represents your like or dislike of that item. You will rate each item on a separate scale.

e.g.

Bread

<p>| | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Dislike extremely  Dislike very much  Dislike moderately  Dislike slightly  Neutral  Like slightly  Like moderately  Like very much  Like extremely

If you have never eaten a particular food, or never experienced one of the listed items, please rate the item as ‘neutral’.

Please turn the page over to begin the questionnaire.
Section One: Grains/Cereals

Listed below are a number of food items. Please rate how much you like or dislike the food by clearly marking a box.

<p>| | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Cornflakes

2. Plain porridge

3. Muesli

4. Wholegrain bread

5. White bread

6. Spaghetti

7. Rice
8. Grains e.g. Cous cous, semolina, barley, polenta

   [Blank]

9. Quinoa

   [Blank]
Section Two: Meat/Meat alternatives

Listed below are a number of food items. Please rate how much you like or dislike the food by clearly marking a box.

10. Beef steak e.g. rump, fillet, sirloin

<table>
<thead>
<tr>
<th></th>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

11. Lamb e.g. roast leg of lamb, chops

<table>
<thead>
<tr>
<th></th>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

12. Pork Products e.g., ham, bacon

<table>
<thead>
<tr>
<th></th>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

13. Chicken e.g. roast, poached, steam

<table>
<thead>
<tr>
<th></th>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

14. Duck

<table>
<thead>
<tr>
<th></th>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

15. Turkey e.g. breast, slices

<table>
<thead>
<tr>
<th></th>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

16. White fish e.g. whiting, snapper, barramundi, flathead

<table>
<thead>
<tr>
<th></th>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>
17. Pink fish e.g. salmon, trout

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

18. Eggs e.g. fried, poached, scrambled

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

19. Beans e.g., chickpeas, kidney beans

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

20. Lentils

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

21. Tofu

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>
Section Three: Fast Foods

Listed below are a number of food items. Please rate how much you like or dislike the food by clearly marking a box.

22. Hamburgers e.g. McDonalds, Burger King/Hungry Jacks

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

23. Hot chips

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

24. Asian takeaway e.g. Thai, Chinese, Indian foods

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

25. Pizza

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

26. Chicken e.g. KFC, Red Rooster, rotisserie

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

27. Toasted sandwich e.g. ham and cheese, tomato and cheese

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

6
Section Four: Dairy

Listed below are a number of food/beverage items. Please rate how much you like or dislike the food/beverage by clearly marking a box.

28. Milk

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

29. Yogurt

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

30. Cheese

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

31. Ice cream

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>
Section Five: Fruit and vegetables

Listed below are a number of food items. Please rate how much you like or dislike the food by clearly marking a box.

32. Apple

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

33. Pineapple

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

34. Melon e.g. rockmelon, honeydew melon, watermelon

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

35. Berries e.g. strawberries, blackberries, raspberries

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

36. Banana

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

37. Orange

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

38. Grapes

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like much</th>
<th>Like extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tomato</td>
<td>Greens e.g., lettuce, spinach, bok choy</td>
<td>Broccoli</td>
<td>Carrot</td>
<td>Capsicum/Peppers (not chilli pepper)</td>
<td>Cabbage</td>
<td>Brussels sprout</td>
<td>Mushrooms</td>
</tr>
<tr>
<td>---</td>
<td>---------</td>
<td>----------------------------------------</td>
<td>----------</td>
<td>---------</td>
<td>--------------------------------------</td>
<td>---------</td>
<td>----------------</td>
<td>----------</td>
</tr>
<tr>
<td></td>
<td>Dislike extremely</td>
<td>Dislike very much</td>
<td>Dislike moderately</td>
<td>Dislike slightly</td>
<td>Neither like nor dislike</td>
<td>Like slightly</td>
<td>Like moderately</td>
<td>Like very much</td>
</tr>
<tr>
<td></td>
<td>Dislike extremely</td>
<td>Dislike very much</td>
<td>Dislike moderately</td>
<td>Dislike slightly</td>
<td>Neither like nor dislike</td>
<td>Like slightly</td>
<td>Like moderately</td>
<td>Like very much</td>
</tr>
<tr>
<td></td>
<td>Dislike extremely</td>
<td>Dislike very much</td>
<td>Dislike moderately</td>
<td>Dislike slightly</td>
<td>Neither like nor dislike</td>
<td>Like slightly</td>
<td>Like moderately</td>
<td>Like very much</td>
</tr>
<tr>
<td></td>
<td>Dislike extremely</td>
<td>Dislike very much</td>
<td>Dislike moderately</td>
<td>Dislike slightly</td>
<td>Neither like nor dislike</td>
<td>Like slightly</td>
<td>Like moderately</td>
<td>Like very much</td>
</tr>
<tr>
<td></td>
<td>Dislike extremely</td>
<td>Dislike very much</td>
<td>Dislike moderately</td>
<td>Dislike slightly</td>
<td>Neither like nor dislike</td>
<td>Like slightly</td>
<td>Like moderately</td>
<td>Like very much</td>
</tr>
</tbody>
</table>
47. Potato e.g., mashed, boiled, roasted (not deep fried chips)

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

48. Vegetable soup e.g., minestrone, pumpkin

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>
## Section Six: Snack foods

Listed below are a number of **food** items. Please rate how much you like or dislike the food by clearly marking a box.

<p>| | | | | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>49. Potato chips (crisps)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dislike extremely</td>
<td>Dislike very much</td>
<td>Dislike moderately</td>
<td>Dislike slightly</td>
<td>Neither like nor dislike</td>
<td>Like slightly</td>
<td>Like moderately</td>
<td>Like very much</td>
<td>Like extremely</td>
<td></td>
</tr>
</tbody>
</table>

| 50. Corn chips e.g. Doritos |   |   |   |   |   |   |   |   |   |   |
|   | Dislike extremely | Dislike very much | Dislike moderately | Dislike slightly | Neither like nor dislike | Like slightly | Like moderately | Like very much | Like extremely |   |

| 51. Savoury biscuits e.g. Shapes, Savoys |   |   |   |   |   |   |   |   |   |   |
|   | Dislike extremely | Dislike very much | Dislike moderately | Dislike slightly | Neither like nor dislike | Like slightly | Like moderately | Like very much | Like extremely |   |

| 52. Nuts e.g. peanuts, cashews, almonds |   |   |   |   |   |   |   |   |   |   |
|   | Dislike extremely | Dislike very much | Dislike moderately | Dislike slightly | Neither like nor dislike | Like slightly | Like moderately | Like very much | Like extremely |   |

| 53. Sweet biscuits e.g. Oreo, Scotch finger, Tim Tam |   |   |   |   |   |   |   |   |   |   |
|   | Dislike extremely | Dislike very much | Dislike moderately | Dislike slightly | Neither like nor dislike | Like slightly | Like moderately | Like very much | Like extremely |   |

| 54. Chocolate |   |   |   |   |   |   |   |   |   |   |
|   | Dislike extremely | Dislike very much | Dislike moderately | Dislike slightly | Neither like nor dislike | Like slightly | Like moderately | Like very much | Like extremely |   |

| 55. Lollies |   |   |   |   |   |   |   |   |   |   |
|   | Dislike extremely | Dislike very much | Dislike moderately | Dislike slightly | Neither like nor dislike | Like slightly | Like moderately | Like very much | Like extremely |   |
56. Cake e.g. banana, carrot, chocolate

Dislike extremely
Dislike very much
Dislike moderately
Dislike slightly
Neither like nor dislike
Like slightly
Like moderately
Like very much
Like extremely
Section Seven: Fats/Oils

Listed below are a number of food items. Please rate how much you like or dislike the food by clearly marking a box.

Imagine this product in cooking/on bread/as you would usually use it.

<table>
<thead>
<tr>
<th></th>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td>57. Butter</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>58. Margarine</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>59. Olive oil</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
</tbody>
</table>
Section Eight: Beverages

Listed below are a number of beverage items. Please rate how much you like or dislike the beverage by clearly marking a box.

60. Water
   □  □  □  □  □  □  □  □  □  □
   Dislike extremely  Dislike very much  Dislike moderately  Dislike slightly  Neither like nor dislike  Like slightly  Like moderately  Like very much  Like extremely

61. Tea
   □  □  □  □  □  □  □  □  □  □
   Dislike extremely  Dislike very much  Dislike moderately  Dislike slightly  Neither like nor dislike  Like slightly  Like moderately  Like very much  Like extremely

62. Coffee
   □  □  □  □  □  □  □  □  □  □
   Dislike extremely  Dislike very much  Dislike moderately  Dislike slightly  Neither like nor dislike  Like slightly  Like moderately  Like very much  Like extremely

63. Cola soft drinks e.g. Coca Cola, Pepsi
   □  □  □  □  □  □  □  □  □  □
   Dislike extremely  Dislike very much  Dislike moderately  Dislike slightly  Neither like nor dislike  Like slightly  Like moderately  Like very much  Like extremely

64. Citrus soft drinks e.g. lemonade, Solo
   □  □  □  □  □  □  □  □  □  □
   Dislike extremely  Dislike very much  Dislike moderately  Dislike slightly  Neither like nor dislike  Like slightly  Like moderately  Like very much  Like extremely

65. Fruit juice e.g. orange, apple
   □  □  □  □  □  □  □  □  □  □
   Dislike extremely  Dislike very much  Dislike moderately  Dislike slightly  Neither like nor dislike  Like slightly  Like moderately  Like very much  Like extremely

66. Red wine
   □  □  □  □  □  □  □  □  □  □
   Dislike extremely  Dislike very much  Dislike moderately  Dislike slightly  Neither like nor dislike  Like slightly  Like moderately  Like very much  Like extremely
67. White wine e.g. sauvignon blanc, riesling, champagne

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

68. Beer e.g. lager, bitter

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>
Section Nine: Other

Listed below are a number of oral sensation items. Please rate how much you like or dislike each item by clearly marking a box.

<table>
<thead>
<tr>
<th>Item</th>
<th>Rating Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>The heat/burn of a spicy meal</td>
<td>Dislike extremely, Dislike very much, Dislike moderately, Dislike slightly, Neither like nor dislike, Like slightly, Like moderately, Like very much, Like extremely</td>
</tr>
<tr>
<td>The bitterness of coffee</td>
<td>Dislike extremely, Dislike very much, Dislike moderately, Dislike slightly, Neither like nor dislike, Like slightly, Like moderately, Like very much, Like extremely</td>
</tr>
<tr>
<td>The sweetness of fairy floss</td>
<td>Dislike extremely, Dislike very much, Dislike moderately, Dislike slightly, Neither like nor dislike, Like slightly, Like moderately, Like very much, Like extremely</td>
</tr>
<tr>
<td>The fatty taste of deep fried foods</td>
<td>Dislike extremely, Dislike very much, Dislike moderately, Dislike slightly, Neither like nor dislike, Like slightly, Like moderately, Like very much, Like extremely</td>
</tr>
<tr>
<td>The coolness of mint toothpaste</td>
<td>Dislike extremely, Dislike very much, Dislike moderately, Dislike slightly, Neither like nor dislike, Like slightly, Like moderately, Like very much, Like extremely</td>
</tr>
</tbody>
</table>
Section Ten: Non-foods

Listed below are a number of non-food items. Please rate how much you like or dislike the item by clearly marking a box.

74. Jumping in a pool on a hot day

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

75. Smell of freshly cut grass

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

76. Glare of headlights

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>

77. Walking barefoot on hot pavement

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like nor dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
</table>
Appendix O
Cancer Council Victoria Food Frequency Questionnaire –
The Dietary Questionnaire for Epidemiology Study Version
2 (Study 1 and Study 2)
For each food shown on this page, indicate how much on average you would usually have eaten at main meals during the past 12 months. When answering each question, think of the amount of that food you usually ate, even though you may rarely have eaten the food on its own.

If you usually ate more than one helping, fill in the oval for the serving size closest to the total amount you ate.

11. When you ate potato, did you usually eat: ○ I never ate potato
   ○ Less than A
   ○ Between A & B
   ○ Between B & C
   ○ More than C

12. When you ate vegetables, did you usually eat: ○ I never ate vegetables
   ○ Less than A
   ○ Between A & B
   ○ Between B & C
   ○ More than C

13. When you ate steak, did you usually eat: ○ I never ate steak
   ○ Less than A
   ○ Between A & B
   ○ Between B & C
   ○ More than C

14. When you ate meat or vegetable casserole, did you usually eat: ○ I never ate casserole
   ○ Less than A
   ○ Between A & B
   ○ Between B & C
   ○ More than C
### Times You Have Eaten

#### Cereal Foods, Sweets & Snacks

<table>
<thead>
<tr>
<th>Item</th>
<th>Never,极少 occasions</th>
<th>1-3 times a month</th>
<th>1 time a week</th>
<th>2 times a week</th>
<th>3 times a week</th>
<th>More than 3 times a week</th>
<th>Per Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Bran®</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sultana Bran®, FiberFlax™, Branflakes®</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weet Bix®, Vita Bix®, Weetabix®</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cornflakes, Nutrigrain®, Special K®</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pottigel</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muesli</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pasta or noodles (include lasagne)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crackers, crispbread, dry biscuits</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweet biscuits</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cakes, sweet pies, tarts and other sweet pastries</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat pies, pasties, quiche and other savoury pastries</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pizza</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamburger with a bun</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chocolate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavoured milk drink (cocoa, Milo®, etc.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanut butter or peanut paste</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn chips, potato chips, Twassticks®, etc.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jam, marmalade, honey or syrup</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegemite®, Marmite®, or Praise®</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Dairy Products, Meat & Fish

<table>
<thead>
<tr>
<th>Item</th>
<th>Never,极少 occasions</th>
<th>1-3 times a month</th>
<th>1 time a week</th>
<th>2 times a week</th>
<th>3 times a week</th>
<th>More than 3 times a week</th>
<th>Per Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheese</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loaf</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuna</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lamb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pork</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ham</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corned beef, dinner meat, or salami</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sausages, Cumberland, or salami</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish, steamed, grilled, or poached</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish, fried, breaded (tahong)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Fruit

<table>
<thead>
<tr>
<th>Item</th>
<th>Never,极少 occasions</th>
<th>1-3 times a month</th>
<th>1 time a week</th>
<th>2 times a week</th>
<th>3 times a week</th>
<th>More than 3 times a week</th>
<th>Per Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tinned or frozen fruit (any kind)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit juice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oranges or other citrus fruit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peaches</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bananas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watermelon, rockmelon (cantaloupe), honeydew, etc.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pineapple</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strawberries</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apricots</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peaches or nectarines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mango or paw paw</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avocado</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Times You Have Eaten

**Vegetables (Including Fresh, Frozen and Tinned)**

<table>
<thead>
<tr>
<th>Vegetable</th>
<th>Never</th>
<th>&lt; 1 Time per Month</th>
<th>1 to 3 Times per Month</th>
<th>4 to 6 Times per Month</th>
<th>7 to 12 Times per Month</th>
<th>More than 12 Times per Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potatoes, roasted or fried (include hot chips)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Potatoes, cooked without fat</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Tomato sauce, tomato paste or tinned tomatoes</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Fresh or tinned tomatoes</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Peppers (capsicum)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Lettuce, endive, or other dark greens</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Cucumber</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Celery</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Beetroot</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Cabbage or Brussel sprouts</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Broccoli</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Silverbeet or spinach</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Peas</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Green beans</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Bean sprouts or lentils, etc.</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Soy beans, soy beans, or tofu</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Other beans (include chick peas, lentils, etc.)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Pumpkins</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Onion or leeks</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Garlic (and garlic-based)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Mushrooms</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

**16.** Over the last 12 months, how often did you drink wine or spirits?

### Times That You Drank

- Beer (low alcohol)
- Beer (full alcohol)
- Cider
- Wine
- Spirit (include sparkling wines)
- Other (named alcoholic drinks, e.g. sherry, port, liqueur, etc.)

Please count the next two questions please count the amount you drank into glasses using the examples given below. For spirits, improve your liquid at the containing wires, please count each glass (50 ml) as one glass.

1 glass of beer = 425 ml
1 large bottle of beer (759 ml) = 4 glasses
1 bottle wine (759 ml) = 6 glasses
1 bottle of port or sherry (759 ml) = 12 glasses

**17.** Over the last 12 months, on days when you were drinking, how many glasses of beer, wine and/or spirits altogether did you usually drink?

<table>
<thead>
<tr>
<th>Number of Glasses per Day</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10 or more</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

**18.** Over the last 12 months, what was the maximum number of glasses of beer, wine and/or spirits that you drank in 24 hours?

<table>
<thead>
<tr>
<th>Maximum Number of Glasses per 24 Hours</th>
<th>1-2</th>
<th>3-4</th>
<th>5-6</th>
<th>7-8</th>
<th>9-10</th>
<th>11-12</th>
<th>13-14</th>
<th>15-16</th>
<th>17-18</th>
<th>19 or more</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

© Copyright The Cancer Council Victoria 2000.

Thank you for completing this questionnaire.

**DO NOT WRITE IN THIS AREA.**

97940
Appendix P
Deakin Food Frequency Questionnaire – Printed Version
(Study 3)
Food Frequency Questionnaire

Background
This questionnaire is designed to estimate your usual pattern of food intake by providing you with information on how often, on average, you consumed certain foods and beverages during the last month.

Confidentiality
All the information provided in this questionnaire will be treated in the strictest confidence.

How to fill in the questionnaire
Fill in the boxes using a cross. Please avoid making any stray marks on the form. Should you need to change an answer, please erase the incorrect mark completely. Please mark one box for every food listed. If you never eat a particular food, fill in the box for ‘Never, or less than once a month’.

<table>
<thead>
<tr>
<th>Example</th>
<th>Average number of times consumed in the last month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pineapple</td>
<td>Never, or less than once a month</td>
</tr>
<tr>
<td></td>
<td>1-3 times per month</td>
</tr>
<tr>
<td></td>
<td>4-6 times per week</td>
</tr>
<tr>
<td></td>
<td>Once per day</td>
</tr>
<tr>
<td></td>
<td>2-3 times per week</td>
</tr>
<tr>
<td></td>
<td>4-5 times per week</td>
</tr>
<tr>
<td></td>
<td>6+ times per week</td>
</tr>
</tbody>
</table>

Please cross only one box per row.

Completion of this questionnaire is voluntary

For each food item, fill in the box that best represents your average pattern of consumption of that food over the previous month. For example:

- If you usually eat two slices of wholemeal toast at breakfast, a sandwich using two slices of wholemeal bread at lunch, and a white roll at dinner time and you usually eat no other bread during the day, fill in the box for 4-5 times per day for wholemeal/mixed grain bread etc and the ‘Once per day’ box for white bread etc.
- If you usually eat a banana at breakfast seven times a week and an apple at lunch three times a week, and you usually eat no other bananas or apples during the week, fill in the box for ‘Once per day’ for banana and the ‘2-4 times per week’ box for apple.

Think about all eating occasions
When reading through the list of foods, please think back over the previous month. Think carefully about foods and beverages consumed away from home and when on holidays, as well as those foods prepared and consumed at home. Also think about foods and beverages consumed on special occasions such as Christmas, Easter and birthdays as well as those you eat more often.

Mixed foods
Some commonly consumed mixed foods, such as salads, stir-fried vegetables etc, have been listed as distinct items. Other foods, such as sandwiches, are not listed as distinct items as their composition varies depending on how they are made up. Think about separate ingredients that make up these foods and answer accordingly. For example:

- If you usually eat a ham and mixed salad sandwich once a week, and you usually eat no other ham or mixed salad during the week, fill in the ‘Once per week’ box for ham and the ‘Once per week’ box for green/mixed salad in a sandwich.
Section one

For each food listed, fill in the box indicating how often, on average, you consumed that food in the past month.

Please fill in a box for each food listed, even if you never eat it.

<table>
<thead>
<tr>
<th>Dairy Foods</th>
<th>1-3 times per month</th>
<th>Once per week</th>
<th>2-4 times per week</th>
<th>5-6 times per week</th>
<th>Once per day</th>
<th>2-3 times per day</th>
<th>4-5 times per day</th>
<th>5+ times per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavoured milk drink (e.g. milkshake, iced coffee, hot chocolate)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Milk as a drink</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Milk on breakfast cereals</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Milk in hot beverages (e.g. in coffee, tea)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Cream or sour cream</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Ice-cream</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Yoghurt, plain or flavoured (including fromage frais)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Cottage or ricotta cheese</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Cheddar and other cheeses</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bread and Cereal Foods</th>
<th>1-3 times per month</th>
<th>Once per week</th>
<th>2-4 times per week</th>
<th>5-6 times per week</th>
<th>Once per day</th>
<th>2-3 times per day</th>
<th>4-5 times per day</th>
<th>5+ times per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>White bread, toast or rolls</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Wholemeal/mixed grain bread, toast</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>English muffin, bagel or crumpet</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Dry or savoury biscuits, crispbread, crackers</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Muesli</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Cooked porridge</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Breakfast cereals</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Rice (including white or brown)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Pasta (including filled), noodles</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Meat, Fish, Eggs</th>
<th>1-3 times per month</th>
<th>Once per week</th>
<th>2-4 times per week</th>
<th>5-6 times per week</th>
<th>Once per day</th>
<th>2-3 times per day</th>
<th>4-5 times per day</th>
<th>5+ times per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mince dishes (e.g. rissoles, meat loaf)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Mixed dishes with beef, veal (e.g. cassoulet, stir fry)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Beef, veal - roast, chop or steak</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Mixed dishes with lamb (e.g. cassoulet, stir fry)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Lamb - roast, chop or steak</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Mixed dishes with pork (e.g. cassoulet, stir fry)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Pork - roast, chop or steak</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Sausage, frankfurter</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Bacon</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Ham</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Luncheon meats, salami</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Liver (including pate)</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Food Category</td>
<td>Frequency Options</td>
<td>1-3 times per month</td>
<td>Once per week</td>
<td>2-4 times per week</td>
<td>5-6 times per week</td>
<td>Once per day</td>
<td>2-3 times per day</td>
<td>4-5 times per day</td>
</tr>
<tr>
<td>---------------</td>
<td>-------------------</td>
<td>---------------------</td>
<td>--------------</td>
<td>-------------------</td>
<td>-------------------</td>
<td>-------------</td>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Mixed dishes with chicken, turkey, duck (e.g. lasagna, stir-fry)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken, turkey, duck - roast, steamed, BBQ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canned tuna, salmon, sardines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish, steamed, baked, grilled</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish, fried</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other seafood (e.g. prawns)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweets, Baked Goods, and Snacks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cakes, sweet muffins, scones, or pikelets</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweet pies or sweet pastries</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other puddings or desserts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plain sweet biscuits</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cream, chocolate biscuits</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat pie, sausage roll or other savoury pastries</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pizza</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamburger</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chocolate (including chocolate bars e.g. Mars bars)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other confectionery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jam, marmalade, syrup or honey</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanut butter, other nut spreads</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegemite, Marmite, Piromite</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nut</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potato chips, corn chips, Twizzles, etc.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dressings</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oil and vinegar dressing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mayonnaise or other creamy dressing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-dairy Beverages</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit juice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetable, tomato juice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit juice drink or fruit drink</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-sugar cordial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cordial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food Item</td>
<td>Never, or less than once a month</td>
<td>1-3 times per month</td>
<td>Once per week</td>
<td>2-4 times per week</td>
<td>5-6 times per week</td>
<td>Once per day</td>
<td>2-3 times per day</td>
<td>4-6 times per day</td>
</tr>
<tr>
<td>-----------</td>
<td>---------------------------------</td>
<td>---------------------</td>
<td>--------------</td>
<td>-------------------</td>
<td>-------------------</td>
<td>-------------</td>
<td>------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Low-salt soft drink, soft drinks (including flavoured mineral water)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water (including unfavoured mineral water, soda water, tap water)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coffee</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soy beverages</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beer - low alcohol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beer - ordinary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red wine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White wine or champagne/sparkling wine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wine cooler</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sherry or port</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spirits, liqueurs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetables (including frozen and tinned)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disguised as salad (including lettuce, tomato, etc.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In a sandwich</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>As a side salad with a main meal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stewed or mixed vegetables</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetable casserole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For each food listed, fill in the box indicating how often as an average you consumed that food in the past month.

Excluding their use in the above mixed dishes, please indicate how often you eat the following vegetables:

- Potato, boiled, mashed, baked
- Hot chips
- Pumpkin
- Sweet potato
- Peas
- Green beans
- Silver beet, spinach
- Broccoli
- Cauliflower
- Brussels sprouts, cabbage, coleslaw
- Carrots
<table>
<thead>
<tr>
<th>Food Item</th>
<th>Never or less than once a month</th>
<th>1-3 times per month</th>
<th>Once per week</th>
<th>2-4 times per week</th>
<th>5-6 times per week</th>
<th>Once per day</th>
<th>2-3 times per day</th>
<th>4-5 times per day</th>
<th>5+ times per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zucchini, eggplant, squash</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capsicum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweetcorn, corn on the cob</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mushrooms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomatoes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kale</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Celery, cucumber</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onion or leeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybeans, tofu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baked beans</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other beans, lentils</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruits (including dried, frozen and tinned)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple or pear</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange, mandarin or grapefruit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banana</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peach, nectarine, plum or apricot</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mango or paw-paw</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pineapple</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grapes or berries</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melon (e.g. watermelon, rockmelon, honey dew melon)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin and Mineral Supplements (including tablets, capsules or drops)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multi vitamin with iron or other minerals</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multi vitamin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin E</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-carotene</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folic Acid/Folate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Section Two

1. What type of milk do you usually consume?
   - Whole
   - Low/reduced fat
   - Skim
   - Evaporated or sweetened condensed
   - None of the above
   - Don't know

2. How often do you use any of the following products?

<table>
<thead>
<tr>
<th>Product</th>
<th>Never/Rarely</th>
<th>Sometimes</th>
<th>Usually</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light cream</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sour light cream</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low/reduced fat ice-cream</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low/reduced fat cheddar-type cheese</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low/reduced fat salad dressing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low/reduced fat spreads</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. How often is the meat you eat trimmed of fat either before or after cooking?
   - Never/Rarely
   - Sometimes
   - Usually
   - Don't eat meat

4. How many serves of vegetables do you usually eat each day?
   (a ‘serve’ = 1/2 cup cooked vegetables or 1 cup of salad vegetables)
   - 1 serve or less
   - 2-3 serves
   - 4-5 serves
   - 6 serves or more
   - Don’t eat vegetables

5. How many serves of fruit do you usually eat each day?
   (a ‘serve’ = 1 medium piece or 2 small pieces of fruit or 1 cup of diced pieces)
   - 1 serve or less
   - 2-3 serves
   - 4-5 serves
   - 6 serves or more
   - Don’t eat fruit

---

0123456789

(1)
0. When cooking, which of the following oils/fats do you use?
   - Olive oil
   - Canola oil
   - Vegetable oil
   - Margarine
   - Dairy blend
   - Lard or dripping
   - Butter

7. How often (do you do/s) add salt to your food after it is cooked. Is it never, rarely, sometimes or usually?
   - Never/rarely
   - Sometimes
   - Usually

8. How often is salt added to your food during cooking. Is it never, rarely, sometimes or usually?
   - Never/rarely
   - Sometimes
   - Usually
   - Don't know

Please enclose this questionnaire in the folder and reply-paid, self-addressed envelope provided.

Thank you for your co-operation.
Appendix Q
List of Collapsed Consumption Variables (Study 3a)
<table>
<thead>
<tr>
<th>FFQ Variable</th>
<th>Collapsed Response Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread and cereal foods: ‘White bread, toast or rolls’, ‘wholemeal/mixed grain bread, toast or roll’, ‘muesli’, ‘breakfast cereal’, and ‘rice (including white or brown)’</td>
<td>1= less than 3 times per month; 2= 1-4 times per week; 3= more than 5 times per week</td>
</tr>
<tr>
<td>Bread and cereal foods: ‘English muffin, bagel or crumpet’, ‘dry or savoury biscuits, crispbread, crackers’, ‘cooked porridge’, and ‘pasta (including filled), noodles’</td>
<td>1= less than 3 times per month; 2= more than once per week</td>
</tr>
<tr>
<td>Starchy vegetables: ‘Potato, boiled, mashed, baked’, ‘Pumpkin’, ‘Sweet potato’, ‘Peas’, and ‘Other beans, lentils’</td>
<td>1= less than 3 times per month; 2= more than once per week</td>
</tr>
<tr>
<td>Sweets: ‘Other confectionary’, ‘Jam, marmalade, syrup, honey’</td>
<td>1= less than 3 times per month; 2= more than once per week</td>
</tr>
<tr>
<td>Sweet non-dairy Beverages: ‘Fruit juice’, ‘Vegetable, tomato juice’, ‘Fruit juice drink or fruit drink’, ‘Low-joule cordial’, ‘Cordial’, ‘Low-Joule soft drink’, and ‘Soft drinks (including flavoured mineral water)’</td>
<td>1= less than 3 times per month; 2= 1-4 times per week; 3= more than 5 times per week</td>
</tr>
<tr>
<td>Fruits (including dried, frozen, and tinned): ‘Apple or pear’, ‘Orange, mandarin, or grapefruit’, ‘Banana’, ‘Peach, nectarine, plum, or apricot’, ‘Grapes or berries’, and ‘Melon (e.g., watermelon, rockmelon, honeydew melon)’</td>
<td>1= less than 3 times per month; 2= more than once per week</td>
</tr>
<tr>
<td>Fruits: ‘Pineapple’ and ‘Mango’</td>
<td>1= less than 3 times per month; 2= more than once per week</td>
</tr>
<tr>
<td>Starchy and fatty foods: ‘Hot chips’, ‘Potato chips, corn chips, Twisties, etc.’, ‘meat pie, sausage rolls or other savoury pastries’, ‘pizza’, and ‘hamburger’</td>
<td>1= less than 3 times per month; 2= 1-3 times per month; 3= more than once per week</td>
</tr>
<tr>
<td>Sweet and fatty foods: ‘Chocolate’</td>
<td>1= less than 3 times per month; 2= 1-4 times per week; 3= more than 5 times per week</td>
</tr>
<tr>
<td>Sweet, starchy, fatty foods: ‘Cakes, sweet muffins, scones or pikelets’, ‘sweet pies or sweet pastries’, ‘other puddings or desserts’, ‘plain sweet biscuits’, ‘cream chocolate biscuits’</td>
<td>1= never or less than once a month; 2= 1-3 times per month; 3= more than once per week</td>
</tr>
</tbody>
</table>
Appendix R
Diet Diary Form (Study 2b)
Diet Diary

Instructions:

Please keep a record of everything you **EAT** and **DRINK** for **4 days – 3 weekdays and 1 weekend day (same week).**

Unless advised otherwise, please maintain your usual dietary (eating) habits whilst filling in this questionnaire.

Please include 3 weekdays records, and 1 weekend record over the same week.

**Filling in the food record:**

- Where possible use kitchen scales to weight the quantity of food consumed
- If weighing food is not possible, please use standard measures to describe how much food you consumed, for example, 1 cup of milk, 2 slices of bread, 1 tablespoon of oil
- Please be specific when writing down foods, *i.e.*, rather than stating that you consumed 1 slice of bread, please include the brand of bread (*Tip Top, Bakers Delight*) and the type (*white, wholemeal*)
- Please remember to include all sauces, dressing, and toppings, *i.e.*, 1 slice of wholemeal bread (*Tip Top*) with 1 tablespoon of crunchy peanut butter (*Kraft*)
- Please include how the food was cooked, when required, *i.e.*, chicken breast (with skin), pan fried with 1 tablespoon of peanut oil
- If you use a recipe, please write it down, and state how much of it you consumed, *i.e.*, half of listed recipe
- Do not forget to include drinks, including tea and coffee, juice and alcoholic beverages. Please include the amount of sugar/sweeteners consumed along with your drinks as well.
<table>
<thead>
<tr>
<th>Meal</th>
<th>Time</th>
<th>Food or Drink</th>
<th>Brand and details</th>
<th>Preparation cooking</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>B/F</td>
<td>7am</td>
<td>Bread</td>
<td>Wholemeal, sandwich, Mighty Soft</td>
<td>Toasted</td>
<td>2 slices</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Egg</td>
<td>Chicken, egg</td>
<td>Fried with canola oil</td>
<td>1 extra large egg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Canola Oil</td>
<td>Coles</td>
<td></td>
<td>1 tablespoon</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pork bacon</td>
<td>Primo, Short cut, rindless</td>
<td>Microwaved, no oil</td>
<td>2 slices, short cut rindless bacon</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tea</td>
<td>Twinnings, 1 teabag, Ginger and lemongrass herbal tea with 2 teaspoons of sugar</td>
<td></td>
<td>500 ml</td>
</tr>
<tr>
<td>Lunch</td>
<td>12pm</td>
<td>Salmon roll</td>
<td>1 x bakers delight wholemeal bread roll, 1 canned smoked salmon (John West), 1 teaspoon of full fat mayonnaise (Kraft). No butter</td>
<td>-</td>
<td>1 roll</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sprite</td>
<td>Sprite</td>
<td>-</td>
<td>600 ml</td>
</tr>
<tr>
<td>Meal</td>
<td>Time</td>
<td>Food or Drink</td>
<td>Brand and details</td>
<td>Preparation cooking</td>
<td>Amount</td>
</tr>
<tr>
<td>------</td>
<td>------</td>
<td>---------------</td>
<td>-------------------</td>
<td>---------------------</td>
<td>--------</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Day 1 (continued)_________________       Date:

<table>
<thead>
<tr>
<th>Meal</th>
<th>Time</th>
<th>Food or Drink</th>
<th>Brand and details</th>
<th>Preparation cooking</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Is this a weekday or weekend day?  Weekday / Weekend
Is this your usual day's intake?  YES/ NO
If not, please explain how it differs:
Appendix S
Non-Nutritive Sweetener Questionnaire
(Study 1 and Study 2)
1. Which type of low-energy sweeteners do you consume
   o None
   o Artificial sweeteners (e.g., Sweet’n Low, Equal, Splenda)
   o Natural low-energy sweeteners (e.g., Stevia – Natvia, Stevia sweet)

2. Over the past 12 months, on average, how often did you consume foods and/or beverages sweetened with artificial sweeteners (e.g., artificially sweetened yoghurt, artificially sweetened lollies, sugarfree mints, Diet Coke, Coke Zero, Diet Sprite, Pepsi Max)?
   o Never
   o Once per month or less
   o 1 to 3 times per month
   o 1 to 2 times per week
   o 3 to 6 times per week
   o Once per day
   o More than once a day

3. Over the past 12 months, on average, how often did you consume foods and/or beverages sweetened with natural low-energy sweeteners (e.g., Stevia sweetened carbonated drinks/cordials, Stevia sweetened lollies, Coke Life)?
   o Never
   o Once per month or less
   o 1 to 3 times per month
   o 1 to 2 times per week
   o 3 to 6 times per week
   o Once per day
   o More than once a day

4. Over the past 12 months, on average, how often did you substitute sugar with artificial sweeteners? (Include artificial sweeteners taken with tea and coffee, etc.)
   o Never
   o Once per month or less
   o 1 to 3 times per month
   o 1 to 2 times per week
   o 3 to 6 times per week
   o Once per day
   o More than once a day
5. Over the past 12 months, on average, how often did you substitute sugar with natural low-energy sweeteners? (Include natural sweeteners taken with tea and coffee, etc.)
   o Never
   o Once per month or less
   o 1 to 3 times per month
   o 1 to 2 times per week
   o 3 to 6 times per week
   o Once per day
   o More than once a day
Appendix T
Three-Factor Eating Questionnaire – Printed Version
(Study 3b)
### Three Factor Eating Questionnaire

Please fill in this questionnaire. For each question, please circle the answer which best represents your eating habits. Circle only one answer for each question. The results of this questionnaire are anonymous.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. When I smell a sizzling steak or see a juicy piece of meat, I find it very difficult to keep from eating, even if I have just finished a meal.</td>
<td>T</td>
</tr>
<tr>
<td>2. I usually eat too much at social occasions, like parties and picnics.</td>
<td>T</td>
</tr>
<tr>
<td>3. I am usually so hungry that I eat more than three times a day.</td>
<td>T</td>
</tr>
<tr>
<td>4. When I have eaten my quota of calories, I am usually good about not eating any more.</td>
<td>T</td>
</tr>
<tr>
<td>5. Dieting is so hard for me because I just get too hungry.</td>
<td>T</td>
</tr>
<tr>
<td>6. I deliberately take small helpings as a means of controlling my weight.</td>
<td>T</td>
</tr>
<tr>
<td>7. Sometimes things just taste so good that I keep on eating even when I am no longer hungry.</td>
<td>T</td>
</tr>
<tr>
<td>8. Since I am often hungry, I sometimes wish that while I am eating, an expert would tell me that I have had enough or that I can have something more to eat.</td>
<td>T</td>
</tr>
<tr>
<td>9. When I feel anxious, I find myself eating.</td>
<td>T</td>
</tr>
<tr>
<td>10. Life is too short to worry about dieting.</td>
<td>T</td>
</tr>
<tr>
<td>11. Since my weight goes up and down, I have gone on reducing diets more than once.</td>
<td>T</td>
</tr>
<tr>
<td>12. I often feel so hungry that I just have to eat something.</td>
<td>T</td>
</tr>
<tr>
<td>13. When I am with someone who is overeating, I usually overeat too.</td>
<td>T</td>
</tr>
<tr>
<td>14. I have a pretty good idea about the number of calories in common food.</td>
<td>T</td>
</tr>
<tr>
<td>15. Sometimes when I start eating, I just can't seem to stop.</td>
<td>T</td>
</tr>
<tr>
<td>16. It is not difficult for me to leave something on my plate.</td>
<td>T</td>
</tr>
<tr>
<td>17. At certain times of the day, I get hungry because I have</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>gotten used to eating then.</td>
<td></td>
</tr>
<tr>
<td>18. While on a diet, if I eat food that is not allowed, I consciously eat less for a period of time to make up for it.</td>
<td>T</td>
</tr>
<tr>
<td>19. Being with someone who is eating often makes me hungry enough to eat also.</td>
<td>T</td>
</tr>
<tr>
<td>20. When I feel blue, I often overeat.</td>
<td>T</td>
</tr>
<tr>
<td>21. I enjoy eating too much to spoil it by counting calories or watching my weight.</td>
<td>T</td>
</tr>
<tr>
<td>22. When I see a real delicacy, I often get so hungry that I have to eat right away.</td>
<td>T</td>
</tr>
<tr>
<td>23. I often stop eating when I am not really full as a conscious means of limiting the amount of food that I eat.</td>
<td>T</td>
</tr>
<tr>
<td>24. I get so hungry that my stomach often seems like a bottomless pit.</td>
<td>T</td>
</tr>
<tr>
<td>25. My weight has hardly changed at all in the last ten years.</td>
<td>T</td>
</tr>
<tr>
<td>26. I am always hungry so it is hard for me to stop eating before I finish the food on my plate.</td>
<td>T</td>
</tr>
<tr>
<td>27. When I feel lonely, I console myself by eating.</td>
<td>T</td>
</tr>
<tr>
<td>28. I consciously hold back at meals in order not to gain weight.</td>
<td>T</td>
</tr>
<tr>
<td>29. I sometimes get very hungry late in the evening or at night.</td>
<td>T</td>
</tr>
<tr>
<td>30. I eat anything I want, anytime I want.</td>
<td>T</td>
</tr>
<tr>
<td>31. Without even thinking about it, I take a long time to eat.</td>
<td>T</td>
</tr>
<tr>
<td>32. I count calories as a conscious means of controlling my weight.</td>
<td>T</td>
</tr>
<tr>
<td>33. I do not eat some foods because they make me fat.</td>
<td>T</td>
</tr>
<tr>
<td>34. I am always hungry enough to eat at any time.</td>
<td>T</td>
</tr>
<tr>
<td>35. I pay a great deal of attention to changes in my figure.</td>
<td>T</td>
</tr>
<tr>
<td>36. While on a diet, if I eat a food that is not allowed, I often then splurge and eat other high calorie foods.</td>
<td>T</td>
</tr>
</tbody>
</table>
Part II
Directions: Please answer the following questions by circling the number above the response that is appropriate to you.

37.) How often are you dieting in a conscious effort to control your weight?

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rarely</td>
<td>Sometimes</td>
<td>Usually</td>
<td>Always</td>
</tr>
</tbody>
</table>

38.) Would a weight fluctuation of 5 pounds [2.3 kg] affect the way you live your life?

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not at all</td>
<td>Slightly</td>
<td>Moderately</td>
<td>Very much</td>
</tr>
</tbody>
</table>

39.) How often do you feel hungry?

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Only at mealtimes</td>
<td>Sometimes between meals</td>
<td>Often between meals</td>
<td>Almost always</td>
</tr>
</tbody>
</table>

40.) Do your feelings of guilt about overeating help you to control your food intake?

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Never</td>
<td>Rarely</td>
<td>Often</td>
<td>Always</td>
</tr>
</tbody>
</table>

41.) How difficult would it be for you to stop eating halfway through dinner and not eat for the next four hours?

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Easy</td>
<td>Slightly difficult</td>
<td>Moderately difficult</td>
<td>Very difficult</td>
</tr>
</tbody>
</table>

42.) How conscious are you of what you are eating?

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not at all</td>
<td>Slightly</td>
<td>Moderately</td>
<td>Extremely</td>
</tr>
</tbody>
</table>

43.) How frequently do you avoid ‘stocking up’ on tempting foods?

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Almost never</td>
<td>Seldom</td>
<td>Usually</td>
<td>Almost always</td>
</tr>
</tbody>
</table>
44.) How likely are you to shop for low calorie foods?

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Unlikely</td>
<td>Slightly unlikely</td>
<td>Moderately likely</td>
<td>Very likely</td>
<td></td>
</tr>
</tbody>
</table>

45.) Do you eat sensibly in front of others and splurge alone?

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Rarely</td>
<td>Often</td>
<td>Always</td>
<td></td>
</tr>
</tbody>
</table>

46.) How likely are you to consciously eat slowly in order to cut down on how much you eat?

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Unlikely</td>
<td>Slightly unlikely</td>
<td>Moderately likely</td>
<td>Very likely</td>
<td></td>
</tr>
</tbody>
</table>

47.) How frequently do you skip dessert because you are no longer hungry?

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Almost never</td>
<td>Seldom</td>
<td>At least once a week</td>
<td>Almost everyday</td>
</tr>
</tbody>
</table>

48.) How likely are you to consciously eat less than you want?

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Unlikely</td>
<td>Slightly unlikely</td>
<td>Moderately likely</td>
<td>Very likely</td>
</tr>
</tbody>
</table>

49.) Do you go on eating bingers though you are not hungry?

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Never</td>
<td>Rarely</td>
<td>Sometimes</td>
<td>At least once a week</td>
</tr>
</tbody>
</table>
50.) **On a scale of 0 to 5**, where 0 means no restraint in eating (eating whatever you want, whenever you want it) and 5 means total restraint (constantly limiting food intake and never ‘giving in’), **what number would you give yourself?**

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eat whatever you want, whenever you want it</td>
<td>Usually eat whatever you want, whenever you want it</td>
<td>Often eat whatever you want, whenever you want it</td>
<td>Often limit food intake, but often ‘give in’</td>
<td>Usually limit food intake, rarely ‘give in’</td>
<td>Constantly limiting food intake, never ‘giving in’</td>
<td></td>
</tr>
</tbody>
</table>

51.) **To what extent does this statement describe your eating behaviour?** ‘I start dieting in the morning, but because of any number of things that happen during the day, by evening I have given up and eat what I want, promising myself to start dieting again tomorrow.’

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not like me</td>
<td>Little like me</td>
<td>Pretty good description of me</td>
<td>Describes me perfectly</td>
<td></td>
</tr>
</tbody>
</table>
Appendix U
Milkshake Hedonic and Appetite Ratings – Printed Version
(Study 3b)
HSN313 Laboratory Hunger and Liking Ratings

Student ID:

Instructions
For each question, please clearly place a vertical line at the point that best represents how you are feeling.

1. How full do you feel?
   
   [_________________________]
   
   Not full at all                                               Extremely full

2. How hungry are you?
   
   [_________________________]
   
   Not hungry at all                                           Extremely hungry

3. How strong is your desire to eat?
   
   [_________________________]
   
   Strong desire not to eat                                    Strong desire to eat

4. How much food could you eat right now?
   
   [_________________________]
   
   Nothing at all                                               The most I have ever eaten

5. Do you feel you could eat a snack right now?
   
   [_________________________]
   
   I could not eat a snack right now                            I could eat a snack right now

6. Do you feel you could eat a full meal right now?
   
   [_________________________]
   
   I could not eat a full meal now                              I could eat a full meal now
Please taste a sip, and rate how much you like or dislike the milk shake by clearly marking a box.

<table>
<thead>
<tr>
<th>Dislike extremely</th>
<th>Dislike very much</th>
<th>Dislike moderately</th>
<th>Dislike slightly</th>
<th>Neither like or dislike</th>
<th>Like slightly</th>
<th>Like moderately</th>
<th>Like very much</th>
<th>Like extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>