Oral fatty acid sensitivity and dietary fat consumption

by

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Excessive consumption of dietary fat contributes to weight gain due to its high energy density and palatability. Fat consumption is regulated by many factors, one which may be the ability to detect fat throughout the alimentary canal during ingestion and digestion. This notion seems likely as the taste system acts as a nutrient-toxin detection system, for example, sweet indicates the presence of carbohydrates, while umami taste indicates the presence of proteins. Emerging evidence supports the existence of a fat specific oral detection system that is activated by fatty acids and conveys the presence of fat in foods. Inter-individual variation in oral fatty acid sensitivity is reported to mirror that of the primary tastes, and may be attributed to genetic, biological, or environmental factors. It has also been reported that fat intake may be poorly regulated in obese people due to attenuated fat detection throughout the alimentary canal, leading to excess consumption of dietary fat and increased predisposition to weight gain.

Recent work has reported associations between detection thresholds for the fatty acids and, an individual's dietary fat intake and body mass index (BMI). Oral sensitivity to fatty acids was reported to be both increased and decreased dependent on the amount of dietary fat consumed over a four week period. Fatty acid sensitivity throughout the gastrointestinal (GI) tract has also been strongly associated with overweight and obesity, with impaired detection of fats in both the oral cavity and GI tract reported in obese individuals. This dysfunction may create an excess consumption of energy, potentially via decreased satiety, although the relationships

and mechanisms are yet to be confirmed. Research in this area relies on taste threshold testing of individuals, however whether an individual's sensitivity remains stable over numerous testing sessions is unknown. Thus, it cannot be undoubtedly stated that diet has an effect on oral sensitivity until the reproducibility and reliability of oral fatty acid thresholds are determined. It is also unknown whether a high-fat meal immediately prior to threshold testing has an effect on oral fatty acid thresholds, or whether changes are only seen in sensitivity after habitual changes to diet. Conversely, dietary intake may not be the only factor influencing one's sensitivity; other considerations, for example the number of taste receptors or papillae on the tongue may also play a role.

The overall aim of this thesis was to assess whether oral fatty acid detection thresholds were reliable, and whether thresholds could be influenced by acute or chronic diet. The objectives of this thesis were: (i) to measure the test-retest reliability of oral fatty acid detection thresholds; (ii) to determine the effect of a high-fat meal immediately prior to threshold testing; and (iii) to assess the effect of a weight loss low-fat diet (25% total energy from fat) and a weight loss portion control diet (25% reduction in total energy intake) in an overweight/ obese population, on oral fatty acid thresholds, fat perception and preferences for regular- and low-fat foods.

In the **first study (Chapter 4)**, 17 subjects (8 males: age 31 ± 2.3 years, BMI 22.9 ± 0.6 kg/m^2, 9 females: age 29 ± 1.8 years, BMI 23.4 ± 0.9 kg/m^2) attended 30 laboratory sessions to determine oral detection thresholds for oleic acid (C18:1),

linoleic acid (C18:2) and lauric acid (C12:0). Taste thresholds were also performed using sucrose (sweet), citric acid (sour), sodium chloride (NaCl) (salty), caffeine (bitter) and monosodium glutamate (MSG) (umami). Each stimulus was evaluated on six occasions using ascending forced choice triangle tests over two days. Diet records were also collected prior to each testing session. Oral fatty acid detection thresholds were determined for all subjects and strong intra-class correlations (ICC) were found for within day and across day testing sessions for C18:1, C18:2 and C12:0. The strongest correlations were found for across day testing for C18:1 (ICC = 0.78, CI = 0.49-0.91), C18:2 (ICC = 0.94, CI = 0.84-0.98) and C12:0 (ICC = 0.80, CI = 0.54-0.92). Strong correlations were also found for sweet, sour, salty, bitter and umami tastes (ICC range: 0.7-0.9). This study demonstrated the high test-retest reliability of oral fatty acid thresholds. It also confirmed the reliability of thresholds for sweet, sour, salty, bitter and umami. The novel findings of this study provide evidence supportive of an oral fatty acid specific detection system.

In the **second study (Chapter 5)**, 32 subjects (15 males: age 49.3 ± 4.8 years, BMI 24.7 ± 0.8 kg/m^2, 17 females: age 31.5 ± 2.8 years, BMI 21.86 ± 0.9 kg/m^2) attended three laboratory sessions to determine the effect of a high-fat meal immediately prior to detection threshold testing for C18:1. In each of the three sessions, subjects were given one of three different types of breakfast; a high-fat frittata (60% fat, 20% carbohydrate, 20% protein), a low-fat frittata (20% fat, 40% carbohydrate, 40% protein) or a macronutrient balanced frittata (33% fat, 33% carbohydrate, 33% protein). Oral fatty acid thresholds were evaluated using ascending forced choice triangle tests on two occasions each day; once one hour post breakfast and then one

hour post the completion of the first threshold test. Consumption of a high-fat, low-fat or balanced breakfast prior to thresholds testing had no significant effect on oral fatty acid detection thresholds (P = 0.213). This study also validated the results of study one in that the reliability of oral fatty acid thresholds remained stable both within and across days. Thus, the present study has provided novel evidence regarding the effect of the macronutrient composition of the meal immediately before threshold testing. This study provides preliminary evidence that the composition of the meal consumed by a subject immediately prior to testing may not impact the accuracy of oral fatty acid thresholds, and it can be speculated that a change in habitual consumption is required before differences in sensitivity will be seen.

In the **third study (Chapter 6)**, subjects (n = 53; age 56.5 ± 1.9 years; BMI 31.0 ± 0.7 kg/m^2) completed a randomised dietary intervention whereby they consumed either a low-fat diet (< 25% dietary fat) or a portion control diet (reduction in energy intake by 25%) for six weeks. Oral fatty acid sensitivity, anthropometry, ability to detect the fat content of custard and preference for regular- and low-fat foods were assessed at baseline and week six. Consumption of both a low-fat diet (n = 26) and portion control diet (n = 27) significantly increased oral fatty acid detection thresholds from baseline to week six (P = 0.014), however there were no significant differences between groups (P = 0.060). Significant increases in subject's ability to perceive the fat content of custard were also observed but only in the low-fat diet group (P = 0.017), however no significant differences between groups were observed (P = 0.423) with directional changes for increased fat perception also seen in the

portion control group. Minimal changes in preferences for regular-fat and low-fat foods were observed in both groups with the only significant change observed for an increased liking for low-fat cream cheese ($P = 0.011$). Both diets significantly reduced weight ($P < 0.001$) and BMI ($P < 0.001$). There were no significant differences in the amount of fat consumed (g) between groups ($P = 0.494$). This study found that both the low-fat and portion control diets significantly increased oral fatty acid sensitivity, suggesting that total fat intake over a period of six weeks, rather than percentage energy from fat may be a key factor modulating oral fatty acid sensitivity. Another important finding was that subject's ability to perceive the fat content of custard was significantly improved after consumption of the six week low-fat diet. While sensitivity and perception increased, preference was not changed and presumably this will be an important next step, as habitual diet is often determined by preference.

Therefore, this thesis has reported that oral fatty acid detection thresholds remain stable and reliable over numerous testing sessions and that detection thresholds are not influenced by acute diet. Additionally, oral fatty acid detection thresholds can be increased by both a low-fat and portion control diet over a six week period, adding to the growing body of evidence for this novel area of research.

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LIST OF ABBREVIATIONS

3-AFC	3-alternate forced choice methodology
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BMI	Body mass index
C12:0	Lauric acid
C18:0	Stearic acid
C18:1	Oleic acid
C18:2	Linoleic acid
C18:3	Linolenic acid
C20:4	Arachadonic acid
C20:5	Eicosapentanoic acid
C22:6	Docosahexanoic acid
C6:0	Caproic acid
CCK	Cholecystokinin
CD36	Cluster of differentiation 36
CHAMPS	Community healthy activities model program for seniors
CVD	Cardiovascular disease
DNA	Deoxyribonucleic acid
DRK	Delayed rectifying potassium channels
EDTA	Ethylenediaminetetraacetic acid
FAT	Fatty acid transporter
GI	Gastrointestinal

GLAST	Glutamate aspartate transporter
gLMS	General labelled magnitude scale
GLP-1	Glucagon-like peptide-1
GPCR	G-Protein coupled receptor
HF	High-fat
ICC	Intra-class correlation
ISO	International Organisation for Standardisation
K^+	Potassium
LF	Low-fat
MSG	Monosodium glutamate
Na^+	Sodium
NaCl	Sodium chloride
NEFA	Non-esterified fatty acid
NPY	Neuropeptide Y
NT	Neurotransmitter
NTPDase 2	Ectonucleoside triphosphate diphosphohydrolase 2
OZ-DASH	Australian dietary approaches to stop hypertension
PLCβ2	Phospholipase Cβ2
PROP	6-n-propylthiouracil
PTC	Phenylthiocarbamide
PUFA	Polyunsaturated fatty acid
PYY	Peptide YY
RF	Regular-fat
ROMK	Renal outer medullary potassium channel

SNP	Single nucleotide polymorphisms
T1R	Taste receptor, type 1
T2R	Taste receptor, type 2
TRPM5	Transient receptor potential channel, type M

CHAPTER ONE

Background

The prevalence of obesity is rising in the developed world and this has been associated with excess consumption of dietary fat (National Health and Medical Research Council, 2006). Fat is consumed in excess due to environmental factors, appetite, satiety and hedonic value (Snoek et al., 2004). Energy intake is regulated by many factors, one of which may be the ability to detect fats and other nutrients during ingestion and digestion. Emerging evidence indicates fat detection may occur in the oral cavity via specific receptors, similar to the other oral nutrient receptors used to detect carbohydrates and proteins (Bachmanov & Beauchamp, 2007) and this new evidence may potentially explain the overconsumption of energy and fat. It has also been reported that fat intake may be poorly regulated in obese people due to a dysfunction in satiety and appetite regulation (Speechly & Buffenstein, 2000), suggesting possible attenuation of fat detection throughout the alimentary canal and therefore, excess consumption of dietary fat and in turn predisposition to weight gain.

The taste system is used to detect the nutritional or toxic quality of foods, for example, sugars which are sweet indicate the presence of carbohydrates, while umami taste indicates the presence of proteins (Bachmanov & Beauchamp, 2007). It seems appropriate that as well as sensing protein and carbohydrate, humans would have mechanisms for sensing fat in the oral cavity (Bachmanov & Beauchamp, 2007). Animal electrophysiological and behavioural studies and human studies have provided evidence in support of oral fatty acid chemoreception, likely linked to the

identification of fats in foods (Chale-Rush, Burgess, & Mattes, 2007a; Gilbertson et al., 2005; Kamphuis, Saris, & Westerterp-Plantenga, 2003). Oral exposure to fat (containing fatty acids), but not fat mimetics (no fatty acids) enhances lipid metabolism (increases in triglycerides (TAG)) after sham feeding, which involves sample mastication and expectoration (Mattes, 2001a). Results support the phenomenon of oral fatty acid nutrient detectors because fat specific enzymes and other digestive mechanisms throughout the gastrointestinal (GI) tract were initiated when fats were exposed to the oral receptors; however no such physiological processes were initiated when protein and carbohydrate based fat mimetics were used (Mattes, 2001a). It was suggested that this may be due to the cephalic phase response, which involves the release of pre-absorptive enzymes and hormones when a food is tasted. This mechanism is thought to optimise nutrient digestion, absorption and metabolism (Crystal & Teff, 2006).

The relationship between oral fatty acid sensitivity and dietary fat consumption has been investigated in animal studies, whereby it was revealed that rats that were orally hypersensitive to fatty acids consumed less dietary fat and gained less weight when exposed to a high-fat diet whereas, orally hyposensitive rats consumed excess fat and rapidly gained weight when fed a high-fat diet (Gilbertson, 1998; Gilbertson, et al., 2005). This study suggests that oral sensitivity to fatty acids may play a role or be a contributing factor to weight gain in animals. More recently in humans, Stewart et al. (2011) investigated the potential relationship between fatty acid detection thresholds and GI tract activity. When comparing lean and obese subjects, it was found that obese subjects had higher detection thresholds and an intra-duodenal infusion of the

fatty acid, oleic acid (C18:1), was associated with reduced stimulation of pyloric motility in obese subjects only (Little & Feinle-Bisset). This suggests that the obese are less able to sense fatty acids in the oral cavity and along the GI tract and are therefore, unable to induce an appropriate signalling response. In this way, oral fat exposure may influence appetite responses, food intake, nutritional status and disease risk (Mattes, 2005). Furthermore, oral fatty acid detectors may act as mediators for this response and an individual's oral sensitivity to fatty acids may result in differences in fatty food consumption. However, the relationship between fatty acid sensitivity and diet remains contentious as it is unknown whether sensitivity dictates dietary intake or *vice versa* and if this is the case, other factors including genetics could play a role. Many gaps remain in the current body of evidence for oral fatty acid sensitivity, therefore the purpose of this PhD thesis was to add to the growing evidence base by investigating the test-retest reliability of the threshold measure and determine the influence of short-term dietary fat intake and long-term dietary fat intake on oral fatty acid sensitivity and preferences for fatty foods.

CHAPTER TWO

Literature review

An abridged version has been published in Nutrients 2013; 5, 1287-1300, 'Functionality of fatty acid chemoreception: a potential factor in the development of obesity?'

2.1 Introduction

Obesity is one of the leading causes of preventable disease contributing to negative health outcomes including cardiovascular disease (CVD), type-2 diabetes and cancer (Swinburn et al., 2011; Wang et al., 2011). It is thought that one of the main contributors to overweight and obesity is excess energy consumption, particularly dietary fat. Dietary fat is consumed in excess due to a number of factors including preference for fats, high palatability and satiety responses (Snoek, et al., 2004). One possible mechanism involved in energy intake regulation is the ability to detect fats and other nutrients during ingestion and digestion. It is thought that the oral cavity acts as a nutrient-toxin detection system which regulates ingestion of macronutrients essential for survival and potential toxic substances that may be harmful. Specific receptors for detection of these macronutrients exist both within the oral cavity and gastrointestinal (GI) tract (alimentary canal). When fats are consumed, they are detected by specific receptors in both the mouth and GI tract and induce the release of specific hormones which slow gastric emptying and suppress energy intake (Cummings & Overduin, 2007; Feltrin et al., 2004). Detection of nutrients, in particular fatty acids along the alimentary canal, can directly affect energy intake which raises the possibility that abnormalities to these nutrient detection mechanisms

may be associated with excess energy intake, and possibly fat intake, conceivably promoting obesity (Blundell & Macdiarmid, 1997; Rolls et al., 1994; Speechly & Buffenstein, 2000; Westerterp, 2006).

2.2 Overweight/ obesity

The global rise in overweight and obesity is a worldwide health concern and in some regions has taken the lead over tobacco as the largest preventable cause of disease burden (Hoad, Somerford, & Katzenellenbogen, 2010). There have been some reports that overweight and obesity in children is plateauing in some populations, while others predict that in the coming decades increases in the prevalence of obesity will continue, enhancing the burden of obesity-related mortality and morbidity (Rokholm, Baker, & Sørensen, 2010; Swinburn, et al., 2011).

There are many factors which contribute to weight gain and the consequential increase in the prevalence of overweight and obesity, including the wide availability of cheap, energy-dense foods (Swinburn, et al., 2011). These types of foods are generally high in fat and overconsumption of these foods has been linked to weight gain (Swinburn, et al., 2011). Although we have a dietary requirement to ingest fat for many purposes including the ingestion of essential fatty acids and the absorption of fat soluble vitamins, the modern day food supply, which is abundant in fat, is different from our hunter gatherer heritage where energy dense foods were scarce (Cordain et al., 2005). At a population level, an excess consumption of dietary fat is one of multiple causal factors in the development of overweight and obesity (Bray, Paeratakul, & Popkin, 2004).

2.3 Health effects of excess fat consumption

Although fats are essential for human functioning, excess fat consumption has been linked to numerous negative health effects, for example, CVD, obesity, type-2 diabetes, hypertension and many cancers (Eden & Noakes, 2003). Fat consumption is excessive (greater than 35% total energy from fat) in the overweight and obese population and this is greatly impacting on the health of many populations (Australian Bureau of Statistics, 2008; Miller, Lindeman, Wallace, & Niederpruem, 1990). Diet-induced obesity is due to long-term energy imbalance, where energy intake is greater than energy output (Swinburn, et al., 2011). Excess fat intake is often associated with the development of obesity and several studies have shown that fat intake is positively correlated to the fat mass of subjects with overweight and obese subjects consuming greater than 40% total energy from fat (Dreon et al., 1988; Miller, et al., 1990; Romieu et al., 1988; Tucker & Kano, 1993). However, for the overweight and obese population, a decrease of 5-10% body weight via dietary changes, for example, decreasing total energy intake, specifically energy dense macronutrients like fat, can have large positive effects on an individual's health outcomes suggesting that losing enough weight to return to a healthy body mass index (BMI) range will have even greater health benefits (Diabetes Prevention Program Research Group, 2002). In contrast, a recent meta-analysis has provided novel ideas in that excess weight gain to a certain extent may in fact be protective against disease (Flegal, Kit, Orpana, & Graubard, 2013). Individuals categorised as overweight according to BMI categories (BMI > 25 and ≤ 29.9 kg/m^2) had a 6% lower risk of death than those categorised as normal weight (BMI > 18.5 and ≤ 24.9 kg/m^2) and those classified as grade 1 obese (BMI > 30 and ≤ 35 kg/m^2) had a 5%

lower risk of death than normal weight individuals (Flegal, et al., 2013). These are novel findings which suggest that BMI categories may need revising with regards to disease risk. Although this study provided new insight into disease risk and BMI categorisation, it did however agree with previous research in regards to grade 2 obese individuals (BMI $\geq$ 35 kg/m^2) finding that they were at a 29% increased risk of disease than normal weight individuals (Flegal, et al., 2013). Other studies have also failed to find a link between dietary fat consumption and body mass (Forouhi et al., 2009; Tucker, Seljaas, & Hager, 1997), therefore BMI remains a contentious issue in regards to disease risk and prevention.

In 2008, an estimated 1.46 billion adults were overweight (BMI > 25 kg/m^2) and 502 million adults were obese (BMI > 30 kg/m^2) worldwide (Swinburn, et al., 2011). In Australia 2007-08, 25% of the adult population were obese, 37% were overweight, 37% were normal weight and 2% were underweight (Australian Bureau of Statistics, 2008). The highest rates of overweight and obesity were in the 65-74 year old age bracket at 79% (Australian Bureau of Statistics, 2008). It was also reported that more males (68%) were overweight than obese females (55%) (Australian Bureau of Statistics, 2008). The financial burden of the obesity epidemic in Australia is considerable with obesity related disease estimated to have cost $8.283 billion in 2008 alone (Australian Bureau of Statistics, 2008).

2.4 Factors affecting fat consumption and susceptibility to weight gain

2.4.1 Dietary factors

The environment in which we live can have a major influence on what we eat. High-fat, high-energy dense products are now more readily available to consumers making these food choices cheap and convenient (Prentice & Jebb, 2003; Swinburn, et al., 2011). There have been numerous changes to the food environment over the past 100 years with the introduction of refrigeration and other processing techniques which have allowed the creation and storage of dairy products, cereals, refined sugars and fatty meats (Cordain, et al., 2005; O'Sullivan et al., 2011). These products have changed the diet of humans dramatically from the original hunter gatherer diet which was low in total fat and high in protein (Cordain, et al., 2005; O'Sullivan, et al., 2011). The Western diet now contains excessive amounts of saturated fats and trans fats (above the 10% daily recommendation), putting the population at an increased risk of disease (World Health Organisation, 2000). The major sources of saturated fats in the diet are fatty meats, baked goods, cheese, milk, margarine and butter (Cordain, et al., 2005; O'Sullivan, et al., 2011). In comparison to the Western diet of today, the hunter gatherer diet would have only contained fatty meats, so the consumption of baked goods, cheese, milk, margarine and butter has increased the fat content of diets consumed by those in developed countries (Cordain, et al., 2005). However, despite the potency of this obesogenic environment, not all of the population become obese, suggesting that some are susceptible to weight gain, while others are resistant or protected (Blundell et al., 2005). Susceptibility may be due to genetic, physiologic and metabolic, behavioural and psychological factors. Reasons

for these differences may be due to variation in the appetite regulating process via physiological signalling, metabolic processes or physiological responsiveness to an environmental trigger (Blundell, et al., 2005). The responses to these triggers may be variable, which reflects inter-individual variation in the biological response and is of great interest to nutritionists, physiologists and clinicians as these differences may be an underlying factor for the predisposition of obesity.

2.4.2 Appetite and preference

Chronic overconsumption of food may be due to patterns in eating behaviour, the sensory or hedonic properties which guide behaviour, or the sensations associated with consuming food or following consumption (Blundell, et al., 2005). These factors can be described as behavioural risk factors and may include preference for fatty foods, weakened satiety response (post-ingestive inhibition of further eating), strong oro-sensory preferences, for example, sweetness combined with fattiness, potential for binge eating and a high food induced pleasure response which all may lead to a risk of overconsumption (Blundell, et al., 2005). Vulnerability to any one of these factors may result in susceptibility to weight gain through changes in behaviour. Nonetheless, these factors are unlikely to lead to weight gain alone, but instead are exacerbated by the modern food environment which promotes excessive food consumption and in turn nurtures weight gain and obesity. Characterisation of high-fat and low-fat consumers has been attempted in previous research by dividing young male subjects into behavioural phenotypes with those who consumed high proportions of fat classified as the high-fat phenotype and those consuming the government recommendations for fat intake as the low-fat phenotype (Cooling &

Blundell, 1998). The study highlighted the fact that the males with the high-fat phenotype consumed more fat, as well as total energy intake and a lower percentage carbohydrate intake. Interestingly, they consumed more dairy products, meat, fish and alcohol, but less cereals, bread, fruit and vegetables than the low-fat phenotype (Cooling & Blundell, 1998). The high-fat phenotype group were also more prone to overconsumption of fat in a test meal situation compared to the low-fat phenotype, who did not over consume at the test meal (Cooling & Blundell, 1998). Although the high-fat phenotype showed behaviours that promoted overconsumption and positive energy balance, there was no weight gain in this group suggesting that partial protection to maintain energy balance is occurring. Reasons as to why this is the case may be due to the high-fat phenotype having a higher basal metabolic rate, a higher fat oxidation rate as well as higher fasting plasma leptin (Cooling, Barth, & Blundell, 1998). As to why the low-fat phenotype consumed less fat can only be speculated about with possibilities including behavioural, cognitive or physiological factors (Cooling & Blundell, 2001). However, it is important to keep in mind that this study only used young male subjects, therefore, cannot necessarily be applied to the general population.

Post-ingestive effects of fat including feelings of contentment also promote long-term preference and positive reinforcement (Abumrad, 2005). In other words, when fats are consumed, they produce feelings of pleasure and satisfaction, which cause these feelings to become positively associated with the fatty foods and over a period of time, these foods become preferred (Abumrad, 2005). These effects are not seen with equally palatable, but non-digestible fat substitutes, suggesting it is the response

from the feedback mechanisms of the fatty foods and the dopamine produced in the brain in response to the digestion, that causes the positive associations (Abumrad, 2005; Drewnowski & Bellisle, 2007). This illustrates that the digestion of fats produces feelings of contentment that cannot be produced by fat substitutes. Added to this is that high-fat diets are usually more appealing than low-fat diets due to the high palatability of fat (Drewnowski, Kurth, Holden-Wiltse, & Saari, 1992). Nonetheless, not all individuals over-consume fat when it is available and variation in fat preferences and consumption patterns may have genetic foundations (Liang et al., 2012). Previous reports have indicated that obese subjects have a stronger preference for high-fat foods compared with leaner subjects (Drewnowski, et al., 1992; Mela & Sacchetti, 1991; Rissanen et al., 2002; Salbe et al., 2004; Villarino, Fernandez, Alday, & Cubelo, 2009). However, not all studies agree with this with other finding no associations (Alexy et al., 2011; Cox et al., 1999; Cox et al., 1998). Thus, it remains unclear whether a preference for fats is a predisposing factor for obesity and weight gain.

Fat contributes to the texture, flavour and aroma of a variety of different foods and is usually found in foods that are highly palatable (Drewnowski, 1997). Highly preferred foods can drive consumption regardless of energy deficit or perceived hunger (Sørensen et al., 2003) and can stimulate appetite and eating rate which can lead to overconsumption (Yeomans, Gray, Mitchell, & True, 1997; Yeomans, Lee, Gray, & French, 2001; Yeomans, Tovey, Tinley, & Haynes, 2004). Strategies to alter an individual's inherent preference for fatty foods are an important factor to consider in regards to weight management as changing preference may in turn change

consumption. Previous work has highlighted this concept finding that the consumption of a low-fat diet over 12 weeks can decrease the preference for full-fat foods over low-fat foods, making the low-fat foods preferable over the previously preferred full-fat foods (Mattes, 1993). This suggests that changes in dietary fat consumption over an extended period of time have the potential to alter an individual's preference for fatty foods, which may increase an individual's chance of compliance to a low-fat diet. Furthermore, studies have found a link between preference and intakes for high-fat foods amongst obese people (Drewnowski, et al., 1992; Mela, 1996; Mela & Sacchetti, 1991) suggesting these individuals are or have become more susceptible to the high-fat environment, and therefore adapted to the physiological and post-ingestive effects of fat and in turn, increased consumption and preference for fats which has promoted obesity. Nevertheless, whether or not habitual fat intake can alter the oro-sensory perception of fats or *vice versa* is still unclear.

2.4.3 Genetic factors

Variations in genes can also have an impact on predisposition to overweight and obesity and dietary fat consumption (Bouchard, 2008). When an environmental condition permanently changes, selection of genetic traits is directional and the average population genome is moved to a new set point (Bouchard, 2008; Cordain, et al., 2005; Swinburn, et al., 2011). For example, as our diets have changed from hunter gatherer diets with low amounts of fat to consuming modern processed foods with higher quantities of fats, the genome may slowly adapt to cope with this change, but these changes would take thousands of years to occur (Cordain, et al.,

2005). Alternatively, epigenetic changes occur when changes in phenotype are not attributable to changes in DNA sequencing but in fact due to the environment acting on the phenotype, for example, consumption of a high-fat diet. Examples of such modifications include DNA methylation, which regulates gene expression without altering the underlying DNA sequence. This methylation allows non-genetic factors to cause the individual's genes to behave differently. For example, DNA methylation patterns can be altered by maternal diet; these epigenetic changes can persist for decades (Stein et al., 2007) and possibly be inherited by future generations (Lange & Schneider, 2010).

Every structure throughout the body is determined and controlled by genetics. The number of taste buds and tongue papillae an individual has is controlled by genetics and DNA sequencing. Within these taste buds and papillae are receptors which may help to control food intake. Inter-individual variation in regards to papillae number within the oral cavity is large; some individuals have many tongue papillae, while others have very few (Hayes & Duffy, 2008). Research in mice has found that there may be specific genes which are responsible for determining tongue size and papillae number and size (Reiner et al., 2008). The study examined a number of lingual phenotypes related to ingestion and gustation in a number of genetically-well characterised mice. It was reported that much variation existed in lingual traits, for example, tongue length, width, fungiform papillae number and area and that a significant portion of this variation could be accounted for by gene variants (Reiner, et al., 2008). At this stage, these variants which determine papillae number have not been found to be influenced by diet, but are genetically pre-determined. However,

factors including diet and the environment may be able to switch these genes 'on' or 'off' and therefore, potentially affect the way foods, including fats, are perceived and in turn may control an individual's food/ fat consumption.

Emerging evidence now suggests that dietary fat consumption may be partially regulated by an oral detection mechanism and understanding the functional role of the taste system may be an important factor in understanding reasons for excess energy and fat intake.

2.5 The sense of taste and its function

Taste is a sense that utilises chemoreception for the detection of non-volatile chemicals in potential foods (Bachmanov & Beauchamp, 2007). It is hypothesised that we evolved oral nutrient-toxin detectors (the taste system) to ensure we consume essential nutrients (sugars, fats, amino acids and salts) which are required for functioning and survival, while rejecting foods that may cause harm (Breslin, 2013; Cordain, et al., 2005). Taste qualities including sweet, salty and umami are associated with appetitive responses which, from an evolutionary perspective maximised the chance for consumption of essential nutrients, while aversive responses to excessive sour and bitter tastants maximised the chance of rejection of those foods which may have caused harm (Gilbertson, Damak, & Margolskee, 2000). However, humans seem to be able to tolerate low levels of bitterness (Schifferstein & Verlegh, 1996) and can also learn to enjoy the taste of mildly bitter foods if paired with positive attributes for example in chocolate, coffee or wine (Breslin, 2013).

A taste quality is experienced when the concentration in the oral cavity reaches a level that activates a receptor, which in turn elicits a perception (Keast & Roper, 2007). For example, a compound like sucrose may be in an aqueous solution but at a concentration that cannot be detected. As the concentration of the sucrose increases, the aqueous solution can be discriminated from water and a detection threshold is reached (Keast & Roper, 2007). As the concentration increases further, the recognition threshold will be reached whereby the quality (sweet) will be identified (Keast & Roper, 2007) **(Figure 2.1)**. When a solution is at a sub-threshold concentration, the stimulus is too low to elicit a perception. However, sub-thresholds can enhance the perception of other tastants, for example, when low concentrations of fatty acids have been added to sucrose solutions, preference for the sucrose solution has increased (Gilbertson, et al., 2005; Stratford, Curtis, & Contreras, 2006).

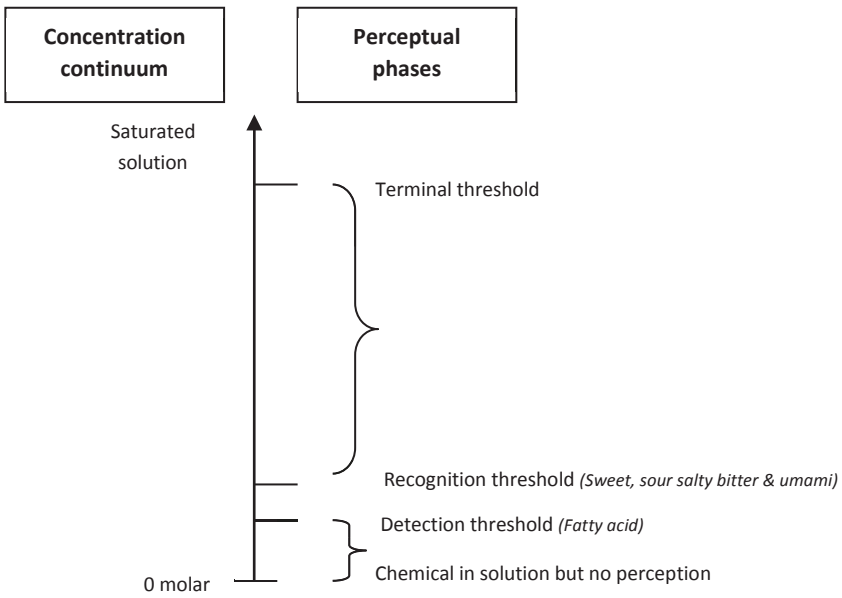


Figure 2.1: The relationship between chemical concentration, detection threshold and recognition threshold. The left-hand side represents chemical concentration from 0 molar solution to a saturated solution. The right-hand side represents the perceptual relationship to increasing concentration and where fatty acid detection is placed in comparison to the five basic tastes (Keast & Roper, 2007).

Within the mouth, three types of cells are believed to express taste receptors. The first type are Type I cells (glial-like cells) which express glutamate aspartate transporter (GLAST), a glutamate transporter, ectonucleoside triphosphate diphosphohydrolase 2 (NTPDase2), a plasma membrane bound nucleotidase that hydrolyses adenosine triphosphate (ATP), renal outer medullary potassium channel (ROMK), a potassium (K^+) channel which may be involved in taste cell homeostasis and ionic currents and the perception of salty taste (Bachmanov & Beauchamp,

2007). The next type are Type II (sensory receptor cells) and these cells house the G-protein coupled receptors (GPCRs) which mediate sweet (taste receptor, type 1 (T1Rs)), umami (T1Rs), bitter (taste receptor, type 2 (T2Rs)) and the downstream signalling molecules (α-gustducin, phospholipase Cβ2 (PLCβ2)), as well as K^+ and sodium (Na^+) channels (Bachmanov & Beauchamp, 2007). Lastly, Type III (pre-synaptic cells) are suspected to form synaptic junctions with nerve terminals and express a number of neuronal like genes, some of which are involved in sour taste perception (Bachmanov & Beauchamp, 2007).

2.6 Possibility of oral fatty acid chemoreception: but fat taste?

Emerging evidence in both animals and humans suggests the existence of oral fatty acid chemoreception mediated via receptors located on taste cells (Laugerette et al., 2007). Taste in the traditional sense arguably requires an effective class of stimuli, a taste cell specific transduction mechanism, activation of gustatory nerves by a peripheral taste mechanism and be perceptually distinguishable from other taste stimuli (Mattes, 2011a). Fatty acids most probably satisfy three of the four criteria, but appear to have no discernible quality (*e.g.,* sweet) associated with them. It may be that the taste system has receptors for compounds such as fatty acids, yet the functional response is not a perception, but rather signalling physiologic processes regarding nutrient uptake or toxin expulsion independent of a perception. In this way the perceptual taste system may be a subset of a larger oral chemoreception system that responds to a wider selection of compounds than historically thought. These affective responses to foods partially drive food consumption and individual variation in affective response may influence overconsumption of foods and be a

factor in the development of diet-related disease such as obesity. Of importance in this debate is emerging evidence indicating a sixth taste quality responsive to fats, a key macronutrient linked with obesity. Viewing the sense of taste as a component of a larger inter-related system including chemesthesis, has previously been postulated by Gibson (1967) and later extended upon by Green (2003). What follows below is a review of evidence for oral fatty acid detection.

2.6.1 Animal evidence for oral fatty acid detection

Animal electrophysiological and behavioral studies have provided evidence in support of oral detection of fatty acids (Fukuwatari et al., 2003; Gilbertson, et al., 2005; Gilbertson, Liu, York, & Bray, 1998; Hiraoka, Fukuwatari, Imaizumi, & Fushiki, 2003; Laugerette et al., 2005; Matsumura et al., 2007; McCormack, Clyburn, & Pittman, 2006; Mindell, Smith, & Greenberg, 1990; Pittman et al., 2008; Primeaux, Braymer, & Bray, 2013; Stratford, et al., 2006; Takeda, Sawano, Imaizumi, & Fushiki, 2001). Gilbertson and Fontenot (1997) investigated the effect of different polyunsaturated fatty acids (PUFA) on K^+ channels directly on the tongues of rats and found that when exposed to linoleic (C18:2), linolenic (C18:3), arachadonic (C20:4), eicosapentanoic (C20:5) and docosahexanoic (C22:6) fatty acids, inhibition of the K^+ channel occurred (Gilbertson & Fontenot, 1997). However, when treated with short-chain fatty acids, no change in K^+ channels was seen, raising the possibility that multiple fatty acid receptor systems may exist in the oral cavity and that stimulation of the taste cells is selective depending on chain length and saturation of the fatty acid (Gilbertson, et al., 1998). Supporting the contention of multiple fatty acid receptors in the oral cavity, recent research in

animals has identified cluster of differentiation 36 (CD36), GPCR120 and GPCR40 on taste tissue (Cartoni et al., 2010; Gotoh et al., 2007; Ichimura et al., 2012; Kawai & Fushiki, 2003; Laugerette, et al., 2005; Matsumura et al., 2009; Naville et al., 2012; Simons, Kummer, Luiken, & Boon, 2010; Zhang et al., 2011). Behavioral studies have been conducted using two-bottle preference tests and have established that healthy rodents show a preference for long-chain PUFA when compared to sensory matched oils, even when they are anosmic, sham-fed and potential confounding factors have been removed including texture, odor and post-ingestive effects (Fukuwatari, et al., 2003; Mindell, et al., 1990; Takeda, et al., 2001; Tsuruta, Kawada, Fukuwatari, & Fushiki, 1999). This suggests that there may be an independent oral mechanism for the detection of fatty acids. In addition, rats that were classified as orally hypersensitive to fatty acids consumed less dietary fat and gained less weight when exposed to a high-fat diet, whereas orally hyposensitive rats consumed excess fat and rapidly gained weight when fed a high-fat diet (Gilbertson, et al., 2005; Gilbertson, et al., 1998). These studies suggest that oral sensitivity to fatty acids may play a role or be a contributing factor to weight gain in animals.

2.6.2 Human evidence for oral fatty acid detection

In humans, several well-controlled studies have been conducted investigating oral detection thresholds for unoxidised fatty acids using sensory matched samples. It was reported that humans could detect C18:2, stearic acid (C18:0), lauric acid (C12:0) and caproic acid (C6:0) in the oral cavity in a water emulsion at threshold concentrations ranging from 0.007% (w/v) to 0.06% (w/v) (Mattes, 2001a, 2009c, 2009d). Similarly, oral detection thresholds have been found for C18:1, C18:2 and

C12:0 when using a stable milk emulsion (Stewart et al., 2010; Stewart, Newman, & Keast, 2011; Stewart, Seimon, et al., 2011). In both studies, non-taste cues were controlled for including (1) textural cues, for example, viscosity, which are normally associated with the mouth feel of fats by the use of mineral oils and gums, (2) olfactory cues through the use of nose clips and (3) visual cues as all tests were conducted under red lights (Mattes, 2009c; Newman & Keast, 2013; Stewart, et al., 2010).

Additional studies have found that oral exposure specifically to fat, but not fat mimetics (replacers) enhances the cephalic response and post prandial triglyceride concentrations (Mattes, 2001a). This finding followed sham feeding (sample mastication with expectoration) of butter and various fat replacers. Furthermore, physiological responses to oral fat exposure included, gastric lipase secretion, altered GI transit, pancreatic exocrine secretions, gut hormone release, mobilisation of stored lipids from enterocytes, pancreatic endocrine secretion and altered lipoprotein lipase activity (Mattes, 2005). Results support the phenomenon of oral fat detection as fat specific enzymes and other digestive mechanisms throughout the GI tract were initiated when fats were exposed to the oral receptors; however no such physiological changes in circulating triacylglycerol concentrations were seen when protein and carbohydrate based fat mimetics were used (Mattes, 2001a). It was believed this was due to the cephalic phase response, which involves the release of pre-absorptive enzymes and hormones when a food is tasted. This mechanism is thought to optimise nutrient digestion, absorption and metabolism (Crystal & Teff, 2006).

Recently, oral fatty acid sensitivity has been measured using a novel method whereby subjects "tasted" edible strips, rather than the previous method of liquid emulsions (Ebba et al., 2012). Although this method is limited by the solubility of fatty acids due to their hydrophobic nature, taste strips may be an effective vehicle for taste recognition due to the low background noise of the strip (Smutzer et al., 2008). In addition, studies have suggested that somatosensory cues may be minimal due to the rapid dissolving time of the strips (Smutzer, et al., 2008). However, this cannot be certain and measures should be put in place to control for texture and odour cues associated with fats. Ebba et al (2012), do not make mention of controlling for these attributes and hence, this may be a limitation of this methodology and its use with fats.

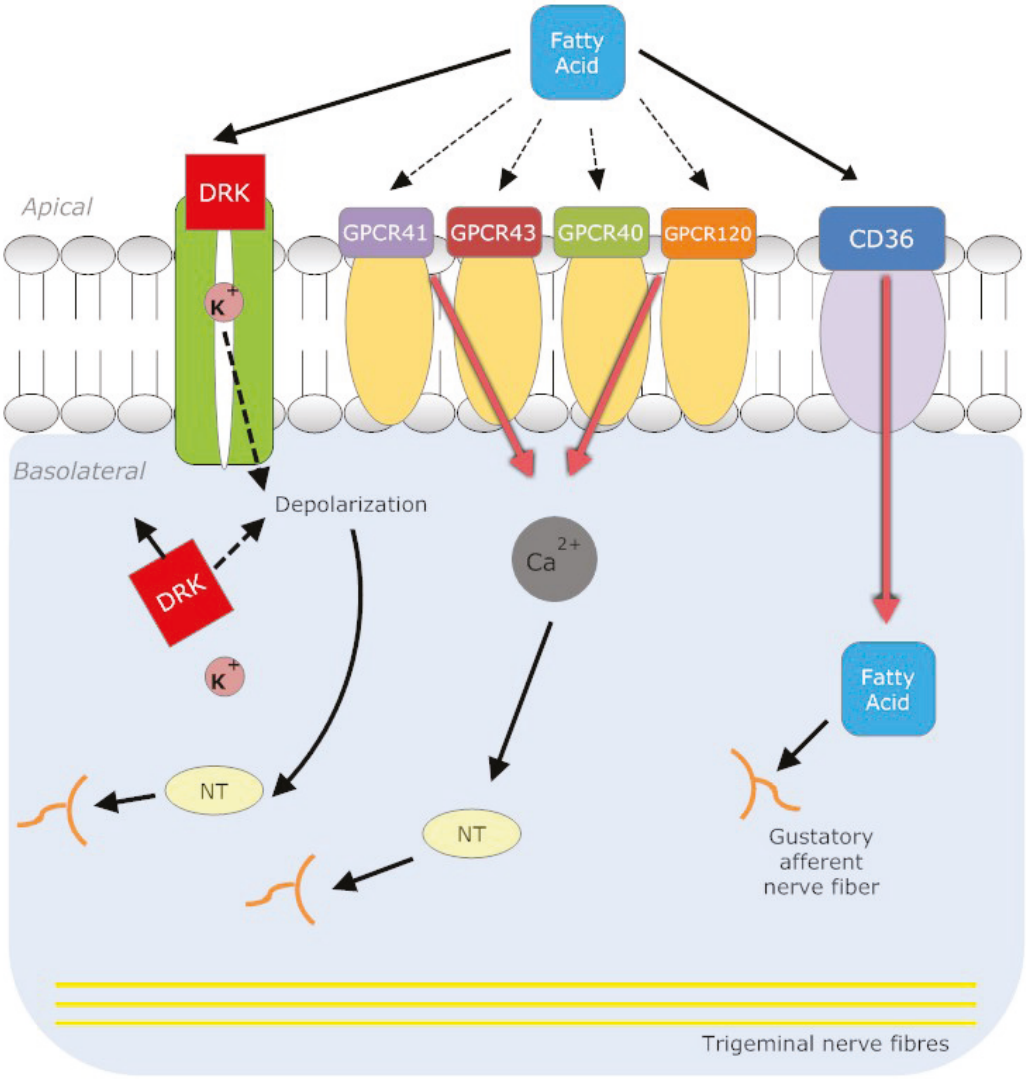
Nonetheless, it is unknown if an individual's oral fatty acid sensitivity as measured by oral detection thresholds remains stable over time, or if the threshold changes as dietary fat intake varies. Studies have confirmed the reliability and reproducibility of taste thresholds for the five prototypical tastes (Ahne, Erras, Hummel, & Kobal, 2000) and our laboratory recently completed similar testing with oral fatty acid thresholds and found results comparable to the prototypical tastes (Newman & Keast, 2013). In contrast, Tucker et al. (2013) compared two types of sensory methodologies (ascending vs. staircase) for the measurement of oral fatty acid detection thresholds and found that subject's thresholds improved after each session (Tucker & Mattes, 2013). Differences in findings may be due to differences in testing methodology (triangle tests with ascending forced choice methodology vs. 3-alternate forced choice (3-AFC) and staircase methodology) and the fact that the

vehicle in which the fatty acids were presented in were different (non-fat milk samples vs. water samples). Both of these studies, however agree that taste thresholds need to be measured more than once to gain an accurate portrayal of one's sensitivity to fatty acids (Newman & Keast, 2013; Tucker & Mattes, 2013).

2.7 Putative mechanisms for fatty acid chemoreception

It is thought that the ability to detect fatty acids is via oral receptors (CD36, GPCRs), ion channels (Delayed Rectify Potassium (DRK) channels) and enzymes (lingual lipase) which have been located in the oral cavity on taste receptor cells within the circumvallate and fungiform papillae (Laugerette, et al., 2007) **(Figure 2.2)**.

Figure 2.2: Putative mechanisms in the gustatory response to fatty acids. Fatty acids may inhibit DRK channels to depolarise the taste receptor cell leading to neurotransmitter (NT) release onto afferent nerve fibres. They may also diffuse through the lingual epithelium where they may affect basolateral DRK channels or trigeminal nerve fibres. Fatty acids may be transported by a GPCR via a second messenger system. In addition, fatty acids may be transported by CD36 through the epithelium onto the afferent nerve fibres (Gilbertson, 1998).



2.7.1 CD36

One of the proposed mechanisms of oral fatty acid nutrient detection is via *CD36* receptors (Abumrad, 2005). CD36 is found in the oral cavity on human taste buds, specifically circumvallate and foliate papillae (Simons, et al., 2010). Results from a mouse study have shown that inactivating the CD36 receptor eliminated a preference for long chain fatty acid (LCFA) enriched solutions and solid foods (Laugerette, et al., 2005). Furthermore, high-fat diet induced rats showed reduced expression of CD36 which may be associated with fatty acid taste adaptation (Zhang, et al., 2011). There is also the possibility that CD36 may be involved with the onset of fat induced satiety (Naville, et al., 2012). This suggests that the *CD36* receptor may play a direct role in fat perception and possibly food regulation (Laugerette, et al., 2005; Pepino, Love-Gregory, Klein, & Abumrad, 2012).

A recent study using obese humans investigated whether oral sensitivity to fatty acids is associated reduced expression of CD36 (Pepino, et al., 2012). Subjects were grouped based on whether or not they were a carrier of the variant rs 1761667-A allele of CD36, which has previously been associated with fat metabolism. Subjects were then assessed for their ability to detect C18:1 in water solutions and those who had the genetic variant of CD36 had lower detection thresholds than those without the variant (Pepino, et al., 2012). The same study also investigated thresholds using both a fatty acid and a triglyceride with and without the addition of orlistat (a lipase inhibitor) and it was found that the inhibition of lipase meant that the release of fatty acids from triglyceride was reduced, therefore diminishing oral fatty acid sensitivity (Pepino, et al., 2012). This study provides strong evidence to suggest CD36 as an

orosensory receptor for dietary fatty acids in humans. Additionally, Keller et al. (2012) has suggested a possible association between polymorphisms in the *CD36* receptor, oral fat perception and fat preference in human subjects (Keller, et al., 2012). These types of physiological signals which detect fat and control consumption are plausible and these mechanisms should be explored further.

2.7.2 GPCRs

The possibility that GPCRs may be involved in fatty acid detection within the oral cavity has been suggested and it is thought that CD36 may work together with other possible receptors like GPCRs in a signalling cascade to detect fatty acids (Galindo et al., 2012). GPCR120 and GPCR40 bind to fatty acids which activate G-proteins leading to the production of the second messenger phospholipase C, and the release of calcium (Liu, Shah, Croasdell, & Gilbertson, 2011). This rise in calcium activates the cation channel Transient Receptor Potential channel type M5 (TRPM5) and allows depolarisation of the receptor potential to occur (Liu, et al., 2011). This potential opens the fatty acid sensitive DRK channels, which are consequently blocked by fatty acids, leading to prolonged depolarisation. This depolarisation is the main driver for the release of neurotransmitters on gustatory afferent fibres (Montmayeur & le Coutre, 2010). GPCR120 and GPCR40 have been expressed in the apical portion of type I and II cells from animal taste buds (Cartoni, et al., 2010; Gotoh, et al., 2007; Matsumura, et al., 2007) and more recently, human taste buds (Galindo, et al., 2012). GPCR120 has been isolated in rat circumvallate, fungiform and foliate papillae (Matsumura, et al., 2007), while GPCR40 are expressed specifically in the circumvallate papillae (Covington, Briscoe, Brown, &

Jayawickreme, 2006). When wild mice were compared to GPCR120 and GPCR40 knock-out mice, the latter showed an attenuated preference for C18:2 and C18:1, suggesting these receptors play a role in the perception of fatty acids (Cartoni, et al., 2010). Furthermore, when GPCR120 deficient mice were fed a high-fat diet, they developed obesity and other side effects of metabolic syndrome indicating a role in regulation of energy intake (Ichimura, et al., 2012).

2.7.3 DRK channels

DRK channels are known to be implicated in the transduction pathway of a variety of taste stimuli. A study by Gilbertson et al. (1998) found that PUFA slow down DRK polarisation on the foliate and circumvallate papillae taste cells and therefore allow fat to be detected (Gilbertson, et al., 1998). In obesity-prone (O-M) rats, greater suppression of the potassium current of DRK channels was seen after dose analysis using C18:2. This suggests prolonged depolarisation of the taste receptors may occur due to suppression of the channel's ability to repolarise following an action potential (Primeaux, et al., 2013).

2.7.4 Lingual lipase

The breakdown products of carbohydrates are sugars and proteins are amino acids both of which have taste activity (Bachmanov & Beauchamp, 2007). Therefore, it could be expected that free fatty acids, the breakdown products of triglyceride would have taste activity too. In animals, lingual lipase is released to cleave triacylglyceride to component fatty acids (Kawai & Fushiki, 2003). Triacylglycerides are too large to be detected or pass through the cell membrane, whereas free fatty acids are able to

translocate through a cellular membrane with ease, however the availability of these in the food matrix is low with fatty foods (including nuts and oils) thought to contain between 0.01%–0.1% non-esterified fatty acids (NEFA) (Kulkarni & Mattes, 2013; Laffargue, de Kochko, & Dussert, 2007). Despite this, it is thought only small quantities of free fatty acids are required to induce a taste response. Consequently, lipase enzymes are very important as they break the triglyceride down so that free fatty acids can be transduced by cellular pathways (Kawai & Fushiki, 2003). Kawai and Fushiki (2003) reported that inhibition of lingual lipase in mice reduces their preference for lipids greatly (Kawai & Fushiki, 2003). This illustrates that in animal models, lingual lipase plays a significant role in oral fat transduction and perception (Kawai & Fushiki, 2003). In humans, however the presence and potential role of lingual lipase is debatable. Data have suggested lipolytic activity may be present in humans, however compared to animal models, activity appears to be weak (Stewart, et al., 2010) and it is yet to be resolved whether sufficient concentrations of lingual lipase are produced or whether this originates from endogenous sources or otherwise. Kulkarni and Mattes (2013) have recently observed mastication of fatty foods (almond butter, almonds, olive oil and shredded coconut) can increase salivary NEFA concentrations from between 20–60 μM compared to control stimulated saliva levels, suggesting these concentrations in foods may be enough to initiate gustatory signalling (Kulkarni & Mattes, 2013). Furthermore, Pepino et al. (2012) investigated the effect of a lipase inhibitor (orlistat) on fatty acid detection in the oral cavity and reported that oral sensitivity was diminished (*i.e.,* increased detection thresholds) when orlistat was present, suggesting that lipase may play a functional role in fatty acid detection in the oral cavity (Pepino, et al., 2012).

There are multiple putative mechanisms to initiate oral fatty acid detectors including CD36 transporter, GPCRs, DRK channels and lingual lipase hydrolysis of fats to fatty acids; however it is yet to be elucidated if/ how these function independently or together in oral fatty acid detection.

2.8 Possible functions of fatty acid chemoreception

Recent research has implicated oral fatty acid sensitivity in weight gain, with those less sensitive (higher taste threshold) having a higher BMI, however not all studies have found the same results (Kamphuis, Saris, et al., 2003; Mattes, 2009d, 2011b; Stewart, et al., 2010; Stewart & Keast, 2012; Stewart, Newman, et al., 2011). Oral fatty acid sensitivity refers to an individual's ability to detect fatty acids in a complex matrix when tasting a solution. The ability to detect fatty acids differs between individuals and variance is likely a result of oral peripheral mechanisms responsible for chemoreception, such as differences in fatty acid receptor functionality or papillae density (Chale-Rush, Burgess, & Mattes, 2007b). Indeed, a positive association between sensitivity and number of taste papillae has been reported (Drayna, 2005; Gilbertson, et al., 2005). Selected studies have found a link between sensitivity to and liking of, or dislike to certain tastants, however myriad factors presumably drive this association (Drewnowski & Henderson, 2000; Keller, Steinmann, Nurse, & Tepper, 2002; Turnbull & Matisoo-Smith, 2002). Thus, further research is required to elucidate the directionality of relationships between oral chemoreception (taste sensitivity), food choice, preferences and taste receptor expression.

Kamphuis et al. (2001) investigated whether there was a link between oral fatty acid sensitivity and BMI (Kamphuis, et al., 2001). The authors suggested the existence of 'fat-tasters' and 'fat non-tasters' reporting that 'fat-tasters' had a lower BMI than 'fat non-tasters', however there was no link between oral fatty acid sensitivity and fat consumption (Gilbertson, et al., 1998; Kamphuis, et al., 2001). Nevertheless, given the low concentration (0.0028% weight/volume (w/v) C18:2) and the fact that only one type of fat was used to determine fat taster status, the existence of 'fat non-tasters' remains controversial. Conversely, a relationship between fat intake and obesity may exist as fat intake is high in the obese population.

The relationship between oral fatty acid sensitivity, BMI and dietary fat intake has recently been investigated and it was reported those who were more sensitive to the fatty acid C18:1 had lower energy intakes and consumed less total dietary fats and saturated fats and were also better at detecting the fat content of food (custard) than less sensitive subjects (Stewart, et al., 2010). This suggests that oral fatty acid chemoreception may be used for the identification of fats. Additionally, individuals who were classified as hypersensitive also had lower BMIs than hyposensitive individuals (Stewart, et al., 2010). Another study by Stewart et al. (2011) extended these results and also found a relationship in humans between fatty acid sensitivity, food consumption and dietary behaviours, whereby those who were hyposensitive consumed more high-fat dairy products, high-fat spreads and fatty red meat (Stewart, Newman, et al., 2011). Conversely, hypersensitive individuals reported behaviors including trimming the fat off meat and avoiding saturated fats (Stewart, Newman, et al., 2011). The exact mechanisms responsible for these differences between hyper-

and hyposensitive groups are not yet understood. In contrast, some studies have reported no associations between oral fatty acid sensitivity and BMI (Kamphuis, Saris, et al., 2003; Mattes, 2009d, 2011b; Newman & Keast, 2013; Stewart & Keast, 2012), with these studies testing oral fatty acid sensitivity on more than one occasion (Mattes, 2009d, 2011b). Reasons as to why there are differences between studies may be due to the fact that the studies which have found associations have used an abbreviated screening procedure for testing oral fatty acid sensitivity based on grouping subjects as hypersensitive and hyposensitive to a specific fatty acid concentration, while the studies that found no associations used a complete threshold testing procedure (*e.g.,* staircase, 3-AFC). Thus, grouping the subjects into hyper- and hyposensitive may be skewing the results. The fact that subject's sensitivity was only measured on one occasion may have resulted in misclassification of sensitivity as it has now been shown that at least two sessions (Newman & Keast, 2013), or seven as recommended by others (Tucker & Mattes, 2013), are needed to minimise the chance of misclassification. In addition, the difference in weight status between the groups in some studies may have not be large enough to detect differences and associations between BMI and oral sensitivity (Newman & Keast, 2013; Tucker & Mattes, 2013). Careful consideration of testing methodology should be made based on the outcomes of the research *e.g.,* using 3-AFC methodology may be more suited to determining hypersensitive versus hyposensitive individuals. Nevertheless, the main outcome of these findings was not to investigate the associations between BMI and oral fatty acid sensitivity, therefore future studies designed specifically to investigate this relationship with greater contrast in BMI are needed. While fatty acid sensitivity may drive fat consumption and preference, the reverse may also be true in

that prolonged fat consumption and/ or preference may be a predictor of sensitivity. In this paradigm, recent research suggests that similarly to the oral cavity, GI responses are attenuated.

2.9 Fatty acid sensitivity in the oral cavity and GI tract

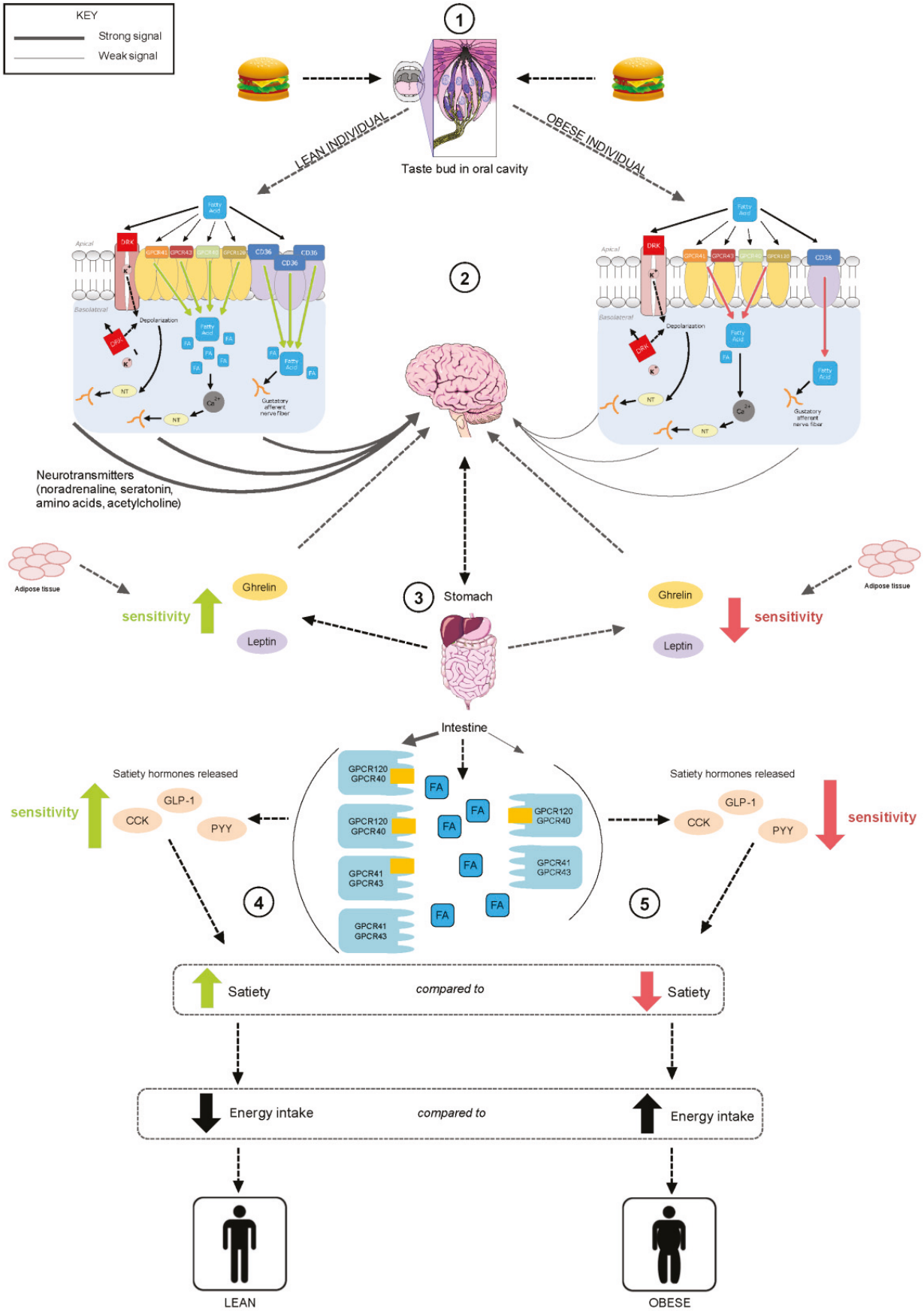
An important mechanism involved in energy intake regulation is the body's ability to detect fat and other nutrients in the oral cavity and GI tract. Evidence now exists that sweet and umami taste receptors for sugars (carbohydrates) and amino acids (proteins) respectively, are co-located in the GI tract (Bachmanov & Beauchamp, 2007), which provides foundation evidence for the hypothesis that the taste system is the first contact for a coordinated alimentary canal nutrient detection system. It is now thought that the same relationship exists for fats, with fatty acid detection occurring in both the oral cavity and GI tract **(Figure 2.3)**.

During digestion, fats have potent effects on hormones that regulate food intake, for example, cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1) decrease gastric emptying and secretion of the hunger stimulating hormone ghrelin, while leptin binds to neuropeptide Y (NPY) to reduce appetite and induce satiety (Feltrin, et al., 2004; Heini et al., 1998). Studies have also suggested that these hormone responses are impaired in obese individuals, raising the possibility that fat intake may be poorly controlled in the obese population due to a dysfunction in appetite regulation **(Figure 2.3)**. When comparing lean and obese individuals after an intra-duodenal infusion of C18:1, no stimulation of pyloric motility occurred in obese individuals, suggesting that they respond differently to the stimulus and that this may

be due to a decreased ability to sense fatty acids along the GI tract (Stewart, Seimon, et al., 2011). Plasma CCK concentrations were also lower in obese subjects after the C18:1 infusion than the lean subjects, however this did not quite reach significance ($P = 0.07$) and could have been due to the small sample size. Additionally, oral fatty acid sensitivity was also impaired in the obese suggesting a coordinated alimentary canal response to fatty acids.

Associations between fatty acid sensitivity status and perceived satiety after a high-fat meal have also been found (Keast, Azzopardi, Newman, & Haryono, 2013). This study presented subjects with four different breakfasts (high-fat, high-carbohydrate, high-protein and macronutrient balanced) and then offered subjects a buffet lunch three hours later. Subjects were grouped as hypersensitive or hyposensitive based on their ability to detect C18:1 and it was found that the hyposensitive individuals consumed more at the buffet meal after the high-fat breakfast than the hypersensitive group. Hypersensitive individuals consumed significantly less energy at the buffet after the high-fat breakfast compared to the high-carbohydrate and macronutrient balanced breakfasts. This suggests that in hyposensitive individuals attenuation of the putative fat detection mechanism may have occurred which may therefore have decreased the effectiveness of the feedback response, in particular the satiety response and in turn, possibly lead to increases energy intake (Keast, et al., 2013).

Figure 2.3: A proposed schematic representation of fatty acid chemoreception in the oral cavity and GI tract (alimentary canal) in lean (left) and obese (right) individuals. (**1**) Fat is present in foods in the form of triglycerides; free fatty acids are generated during the breakdown of fats and by lipase enzymes in the oral cavity. (**2**) Fatty acids access putative receptors (CD36, GPCR 40, 41, 43, 120 and DRK channels) within taste cells; lean individuals have greater quantities of these receptors, compared to obese individuals. The receptors elicit the release of intracellular calcium which in turn activates neurotransmitters and hormones associated with the cephalic response. (**3**) Following fat ingestion, gastric and pancreatic lipase plays a further role in the hydrolysis of fats enabling access to fatty acid receptors on enteroendocrine cells, stimulating satiety hormones and uptake of fatty acids. As a consequence, sensitivity to ghrelin, responsible for hunger stimulation is inhibited, while the satiety inducing hormone leptin is released as are the hormones CCK, Peptide YY (PYY), GLP-1. (**4**) In a lean individual, expression of fatty acid receptors is greater therefore increasing fat sensing ability through the alimentary canal, thereby decreasing energy intake. (**5**) In comparison, obese individuals have decreased expression of fatty acid receptors, attenuating fat sensing ability and increasing energy intake (Newman, Haryono, & Keast, 2013).


KEY
Strong signal
Weak signal
1
Taste bud in oral cavity
LEAN INDIVIDUAL
OBESE INDIVIDUAL
2
Neurotransmitters
(noradrenaline, seratonin,
amino acids, acetylcholine)
Adipose tissue
sensitivity
Ghrelin
Leptin
3
Stomach
Intestine
Satiety hormones released
GLP-1
CCK
PYY
GPCR120
GPCR40
GPCR41
GPCR43
FA
4
5
Satiety
compared to
Energy intake
LEAN
OBESE

2.10 Dietary influences on fatty acid chemoreception

In developed populations, individuals are consuming excess fat which corresponds in many cases to weight gain (Swinburn, et al., 2011), suggesting that adaptation to a high-fat diet may have occurred. Adaptation has been shown to occur with other nutrients including sodium. Increasing the amount of sodium consumed in the diet can increase the taste threshold for NaCl, and therefore increase the concentration of NaCl required to elicit saltiness (Mattes, 1997). This may also be the case for fat because since the 1920s the population's fat consumption has been higher (32%) than the recommended daily levels, peaking in the 1950s with 40-42% of the diet comprising of fat (Nestle & Woteki, 1999). The percentage of fat is also affected by total energy intake, for example, an obese individual may consume 15 MJ of energy per day of which 32% is derived from fat (approximately 130 grams of fat), compared to a lean individual who may consume 7 MJ per day and also 32% from fat (approximately 60 grams of fat). Even though the obese individual is consuming 32% dietary fat of their total energy requirement, they are consuming double the amount of fat than the lean individual, thus as a population we are more obese than any other time in history, and therefore are consuming more fat. Due to the excess consumption of fat, it can be suggested that the body may have adapted to a high-fat environment over the past century, and in turn reduced their sensitivity to fats throughout the alimentary canal, however, the directionality of this relationship is yet to be elucidated (Nestle & Woteki, 1999).

Consumption of a high-fat diet has been shown to decrease oral fatty acid sensitivity (Stewart & Keast, 2012). Dietary influences on the modulation of fatty acid,

specifically C18:1, detection thresholds was investigated whereby subjects were asked to consume a low-fat diet (< 20% fat) for four weeks and after a two week wash out period, then followed a high-fat diet for four weeks (> 40% fat). After the low-fat diet both lean and overweight/ obese subjects' C18:1 detection thresholds decreased, in other words their sensitivity increased and after the high-fat diet thresholds increased, however this only occurred in the lean subjects with no change in thresholds for the obese population after the high-fat diet (Stewart & Keast, 2012). This suggests that in the obese population, consumption of a high-fat diet has occurred that has promoted habituation which therefore decreased the physiological and psychological effects of fat, and in turn decreased oral sensitivity to fat and possibly promoted obesity. However, no differences in dietary fat consumption were seen between the lean and obese group at baseline, therefore, the explanation that the obese population had adapted to the high-fat environment does not provide an explanation as to why the lean group's sensitivity decreased after consumption of the high-fat diet (Running, Mattes, & Tucker, 2013). Additionally, relationships between fatty acid sensitivity and food behaviours have been reported whereby hyposensitive individuals consumed more high-fat dairy products, fatty red meats and fatty spreads compared to hypersensitive individuals (Stewart, Newman, et al., 2011) suggesting that hypersensitive individuals are more likely to engage in fat reducing behaviours than hyposensitive individuals. Associations between dietary fat consumption and oral fatty acid sensitivity remain contentious with some studies finding a relationship between the two (Stewart, et al., 2010; Stewart, Newman, et al., 2011), while others have found no relationship (Mattes, 2009d, 2011b; Stewart & Keast, 2012). Therefore, the relationship between habitual diet and oral fatty acid sensitivity

remains unresolved, however due to the associations between disease risk and fat consumption, this is an area that deserves further investigation.

2.11 Environmental influences on gene expression

The concept of taste sensitivity to and dietary consumption of certain nutrients being genetically predisposed is not novel, for example, it is well established that sensitivity to the bitter compounds phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP) are genetically determined and influence the liking and consumption of brassica vegetables (*e.g.,* brussel sprouts and kale) which contain bitter tasting compounds (*e.g.,* phenols, glucosinolates and terpenes) (Turnbull & Matisoo-Smith, 2002). Differences in oral sensitivity to certain tastants, as well dietary consumption of foods containing these tastants may be due to the amount of receptors present in the oral cavity, or perhaps, the number of taste papillae on the tongue.

As previously mentioned, fats are thought to be detected by fatty acid specific receptors in both the oral cavity and GI tract. The role of these receptors in fat detection is now becoming more apparent with studies highlighting dietary influences on receptor expression. While there is a paucity of evidence in humans, emerging evidence in animals is showing promising findings. Following the consumption of a high-fat diet, CD36 receptor expression on the lingual tissue of rats was reduced highlighting the potential link between fat consumption and CD36 receptor expression (Zhang, et al., 2011). While suggestive at this stage, it does appear that over a short period of time, an individual can adapt to a fatty food

environment. Within a few weeks an individual can adapt to a fatty food environment by becoming less sensitive to the physiological and psychological effects of fat, thereby retaining the ability to consume foods with higher than optimal concentrations of fat (Cordain, et al., 2005). For example, when fed a low-fat diet for 12 weeks, subjects were found to have a decreased preference for high-fat foods, suggesting that adaptation to a low-fat diet had occurred (Mattes, 1993). Consequently, many humans can quickly adapt to the new environment by consuming more fats, but in doing so, the prevalence of obesity is increasing as we have not yet developed a way to deal with the excess fat being consumed. Whether this is a result of changes in gene expression, or perhaps reduced receptor sensitivity remains unresolved.

Similarly, single nucleotide polymorphisms (SNP) (*FTO* rs9939609) have been identified and labelled as important considerations when determining an individual's susceptibility to obesity, with suggestion that impaired satiety, greater food intake and frequent loss of eating control in individuals with at least one risk allele may account for the observed increase in obesity risk (Haupt, 2009; Speakman, Rance, & Johnstone, 2008; Tanofsky-Kraff et al., 2009). Interactions between genetic and dietary factors, in particular dietary fat, may also contribute to the increased susceptibility to obesity (Phillips et al., 2012). Recent research backed up these findings reporting that the gene variant rs9939609 was associated with BMI in the overweight/ obese category and that this may be modulated by saturated fat intake (Phillips, et al., 2012). This highlights how closely associated environmental and genetic factors may be in relation to the development of obesity. However, reported

proportions of BMI variation associated with SNPs have been minimal with less than 10% of variance (difference of ~0.5 kg in the standard deviation of weight) attributed to nucleotide polymorphisms (Gaunt et al., 2001; Yang et al., 2012). Thus, it cannot be undoubtedly stated that there is a direct link between environmental and genetic factors with regards to the predisposition and development of obesity.

2.12 Conclusions

In summary, excess fat consumption is a major cause of obesity as well as other diseases including CVD and type-2 diabetes. Fat intake can be influenced by many factors including habituation and satiety mechanisms and an individual's ability to detect fatty acids in the oral cavity and GI tract via fat specific receptors. There is substantive emerging evidence that an oral nutrient detection system for fatty acids exists in humans. Similar to the five basic tastes, the ability to detect fatty acids in the oral cavity varies amongst the population. This variance may be a factor in influencing one's consumption of high-fat foods, with those who are hypersensitive consuming less fat and preferring low-fat foods than hyposensitive individuals who consume more fat and prefer high-fat foods. It is unknown what the main drivers of this association are, or if in fact reduced fatty acid sensitivity is a predictor of dietary fat consumption, or *vice versa*. Knowing the reproducibility and stability of an individual's oral fatty acid sensitivity is important and needs to be verified. Oral fatty acid sensitivity and its potential links to obesity is a controversial area of research with more investigation needed from a variety of scientific disciplines, but emerging evidence linking oral fatty acid sensitivity with development of obesity is promising. Currently the exact mechanisms associating oral fatty acid sensitivity, fat

consumption and weight gain remain largely elusive. Dietary intake may not be the only factor influencing one's sensitivity; other considerations, for example, the expression of taste receptors in the oral cavity are likely to play an influential role. Investigating oral fatty acid sensitivity and its potential links with dietary fat intake, sensory responses and its potential links with putative fatty acid taste receptor expression will build upon present knowledge; if indeed sensory responses and/or receptor expression can be modulated in response to a high-fat diet, or if oral sensitivity and expression are drivers of fat preference and consequent consumption may provide more of an insight into reasons for excess fat consumption. These ideas are conjectural, but may identify strategies to reduce obesity and related pathologies.

This thesis comprises of seven chapters which are structured as follows:

Chapter One provides an introduction to the topic of this thesis and highlights the key points of focus. **Chapter Two** contains an up-to-date review of the literature in this area including background information on overweight and obesity; fat consumption; the sense of taste; the possibility of oral fatty acid sensitivity; mechanisms of oral fatty acid sensitivity; possible functions of oral fatty acid sensitivity; the link between the oral cavity and GI tract with regards to fatty acid sensing; dietary influences; and environmental influences on gene expression. **Chapter Three** outlines all methodology used in this research which comprised of two sensory evaluation studies and one dietary intervention. **Chapters Four to Six** detail the three studies and includes rationale, methods, results, discussion and conclusion for each. Study 1 (Chapter Four) is a comprehensive investigation of the

test-retest reliability of oral fatty acid detection thresholds. Study 2 (Chapter Five) investigated the influence of a high-fat meal prior to oral fatty acid detection threshold testing. Study 3 (Chapter Six) was a large dietary intervention that compared the effect of a low-fat diet (25% total energy from fat) and a portion control diet on oral fatty acid sensitivity, fat preference and fat perception in an overweight/ obese population. Finally, **Chapter Seven** summarises the findings of the three studies conducted, their limitations and future directions for research.

2.13 Aims

The overall aim of this thesis was to determine the extent to which dietary fat plays a role in determining oral fatty acid sensitivity. The objectives that were critical in addressing the aim were:

- To determine the test-retest reliability of oral fatty acid detection thresholds;
- To measure the effect that a high-fat meal immediately prior to detection threshold testing has on oral fatty acid thresholds;
- To measure the effect that consumption of a low-fat diet (25% total fat) for six weeks has on oral fatty acid detection thresholds in an overweight/ obese population;
- To measure the effect that consumption of a portion control diet for six weeks has on oral fatty acid detection thresholds in an overweight/ obese population.

2.14 Hypotheses

This thesis will test the following hypotheses:

- There will be large inter-individual variation in oral fatty acid detection thresholds;
- There will be minimal intra-individual variation in oral fatty acid detection thresholds, therefore, high test-retest reliability;
- The consumption of a high-fat meal immediately prior to detection threshold testing will not have an effect on oral fatty acid thresholds;
- Consumption of a low-fat diet for six weeks will, significantly increase oral fatty acid sensitivity to C18:1, whereas, consumption of a portion control diet for six weeks may increase oral fatty acid sensitivity, but not to the same extent as those following the low-fat diet.

CHAPTER THREE

Materials, methodology and measurements

3.1 Introduction

This PhD used quantitative methodology to achieve the aims and test the hypotheses described in **Chapter Two**. This Chapter describes all of the methodology employed including sensory evaluation and dietary intervention methods. 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.

3.2 Subjects

Power calculations were performed prior to commencement of all studies to determine suitable sample sizes to achieve statistical power. These calculations are detailed within each chapter. All participants for each study were between the ages of 18-75 years and were non-smokers. For studies one and two, subjects were in good health and for study three, all subjects were overweight or obese at the time of testing. All studies were conducted according to the guidelines laid down by the Declaration of Helsinki and all procedures involving human subjects were approved by the Deakin University Human Research Ethics Committee prior to study commencement. All studies were also registered at *www.actr.org.au* as clinical trials. Written informed consent was obtained from all subjects prior to participation and participants were free to withdraw from all studies at any stage throughout the experiment.

3.2.1 Subject demographics

Prior to each study, subjects were screened for eligibility in which demographic information including weight, height, age, gender and smoking status were collected **(Appendix A)**. In study three, other information collected prior to commencement of the study included food intolerances, medical conditions and use of prescription and non-prescription medications.

3.2.2 Reimbursement

Subjects were reimbursed for their time by means of vouchers/ gift cards from the Coles Group and Myer (department store). Values for gift cards were calculated on an hourly basis: $10 per day of sensory testing during study one, and $16 per week for study three (this was a six week dietary intervention). Subjects who participated in study two were not reimbursed via vouchers/ gift cards.

3.3 Sensory testing

3.3.1 Sensory testing

All sensory testing was performed in individual sensory booths in the sensory laboratory at the School of Exercise and Nutrition Sciences, Deakin University, Melbourne, Australia. Participants were asked to refrain from eating, drinking or chewing gum for at least one hour prior to testing. The individual dietary counselling sessions (study three) took place in a private room at Deakin University under the supervision of the coordinating dietitian of the study (S.T.).

3.3.2 Oral fatty acid solutions – materials and methods

Subjects were examined for their ability to detect low concentrations of fatty acids including oleic acid (C18:1), linoleic acid (C18:2) and lauric acid (C12:0) during study one, which explored the test-retest reliability of oral fatty acid detection thresholds. Detection thresholds for C18:1 were also determined for studies two and three. In doing so, subjects were required to distinguish differences in taste between samples containing fatty acids and samples without fatty acids (control). To prepare the fatty acid solutions, food grade fatty acids; C18:1 and C12:0 (Sigma Aldrich, Missouri, USA) and C18:2 (Larodan Fine Chemicals, Malmö, Sweden) were added to long-life non-fat milk (Devondale, Cobram, Victoria, Australia) at varying concentrations. To minimise textural differences that the fatty acids may have created, all samples were mixed with gum acacia (Deltagen Australia, Boronia, Victoria, Australia) and liquid paraffin (Faulding Remedies, Virginia, Queensland, Australia). To prevent oxidation of fatty acids, ethylenediaminetetraacetic acid (EDTA) (Merck, Darmstadt, Germany) was added to all samples.

Non-fat milk was used as the vehicle for fatty acid solutions as it has been used in prior research conducted in the Deakin University Sensory Laboratory (Stewart, et al., 2010; Stewart, Newman, et al., 2011). For all solutions, oil in water emulsions were created, with the addition of 5% *(w/v)* gum acacia and 5% *(w/v)* mineral oil to reduce potential non-taste cues, for example, viscosity or other textural attributes (Chale-Rush, et al., 2007a). The textural attributes, odour and appearance of fats are easily distinguishable and are responsible for the perception of fat within a food. Therefore, as this thesis is focusing on taste only, it was important to ensure that

these attributes were controlled for between fatty acid solutions and control solutions by the use of mineral oils, gum acacia, nose clips and red lighting. Fatty acids are also vulnerable to oxidation, therefore it was vital to add EDTA to the fatty acid solutions to prevent oxidation. Previous studies have shown that addition of 0.01% *(w/v)* EDTA can delay oxidation of polyunsaturated fatty acids for up to 24 hours (Chale-Rush, et al., 2007a; Stewart, et al., 2010). A stable emulsion was created by combining 100 ml non-fat milk with 5 g gum acacia and 0.01 g of EDTA, then homogenising on a Silverson L4RT homogeniser (Longmeadow, Massachusetts, USA) for 30 seconds/ 100 ml. In a separate beaker, 5 ml of paraffin oil and the set concentrations of fatty acid were combined; 0.02, 0.06, 1.0, 1.4, 2.0, 2.8, 3.8, 5.0, 6.4, 8.0, 9.8, 12.0, 20.0 mM. The concentrations of fatty acids were based on those used in previous research and in the Deakin University Laboratory (Chale-Rush, et al., 2007a; Stewart, et al., 2010). The non-fat milk and gum mixture was then added to the paraffin and fatty acid mixture. Samples were then re-homogenised at 12 000 rpm for 30 seconds/ 100 ml. All samples were freshly prepared on each day of testing and C18:1 and C18:2 were served at room temperature, while C12:0 was heated to 50°C to ensure it was in a liquid state. Control samples were prepared in the same way, without the fatty acids.

3.3.3 Oral fatty acid solutions – threshold measurement

All sensory testing was conducted in a quiet specialised sensory testing facility comprising of seven individual booths. Each participant was separated by vertical dividers and there was no interaction between individuals. Participants were navigated through the procedure by both written and verbal instructions from the

researcher. All subjects were asked to refrain from eating, drinking or chewing gum for at least one hour prior to each testing session.

Oral fatty acid sensitivity was determined via detection thresholds, which are the lowest concentration of stimulus needed to elicit a sensation, using ascending forced choice triangle test sensory methodology **(Figure 3.1)**. During this testing procedure, subjects were presented with three 15 ml samples on a tray; two control samples and one sample containing a certain concentration of fatty acid. The order in which samples were presented was randomised throughout the procedure, for example, if the control samples were labelled A and the fatty acid samples as B, samples could be presented as AAB, BAA or ABA. Samples containing fatty acids were presented in ascending order starting from the lowest concentration (0.02 mM) to the highest (20 mM) (Stewart, et al., 2010). Each sampling cup was labelled with a random 3-digit blinding code which was used by the researcher to ascertain the different samples. Subjects were required to rinse their mouth out with water before beginning the test and between each set of samples. Subjects were then asked to complete the test and were told to place a small amount of solution in their mouth, swish the sample around in their mouth making sure it reached the sides and back of their tongue and then expectorate. Whilst the sample was in their mouth, subjects were asked to focus on the specific taste of that sample and after expectoration, wait 20-30 seconds before tasting the next sample. Subjects were advised to start with the sample on the left and move through to the right of the tray until all samples had been tasted. Once each sample had been tried, subjects were able go back through the samples, repeating the tasting procedure, until they thought they could identify

the sample that was different. If subjects could not perceive a difference between samples, they were asked to pick one out of the three before the next set of samples could be presented. Once a decision was made by the subject, the tray was then returned to the researcher who then determined whether or not the subject had correctly identified the odd sample out of the three. A new set of samples was then presented to the subject dependent of which sample they had chosen as the different one. If they had correctly identified the odd sample out, they were given a second tray which contained the same concentration of fatty acid as the first tray. If they again chose correctly, a third tray was presented with the same concentration of fatty acid on it. However, if they incorrectly identified the odd sample, they were given another tray with the next highest concentration on it. This procedure continued in an ascending manner until the subject could correctly pick the odd sample out in three consecutive sample sets. The chance of correctly guessing the sample with the added fatty acid three consecutive times is 3.7%. Subjects were not aware of the outcome of each test, for example, if they correctly identified the odd sample, they were not made aware of this. Each subject was tested in a separate sensory booth and answers were collected by the researcher **(Appendix B)**. To minimise confounding factors from non-taste sensory inputs, all testing was performed under red lighting and nose clips were worn at all times.

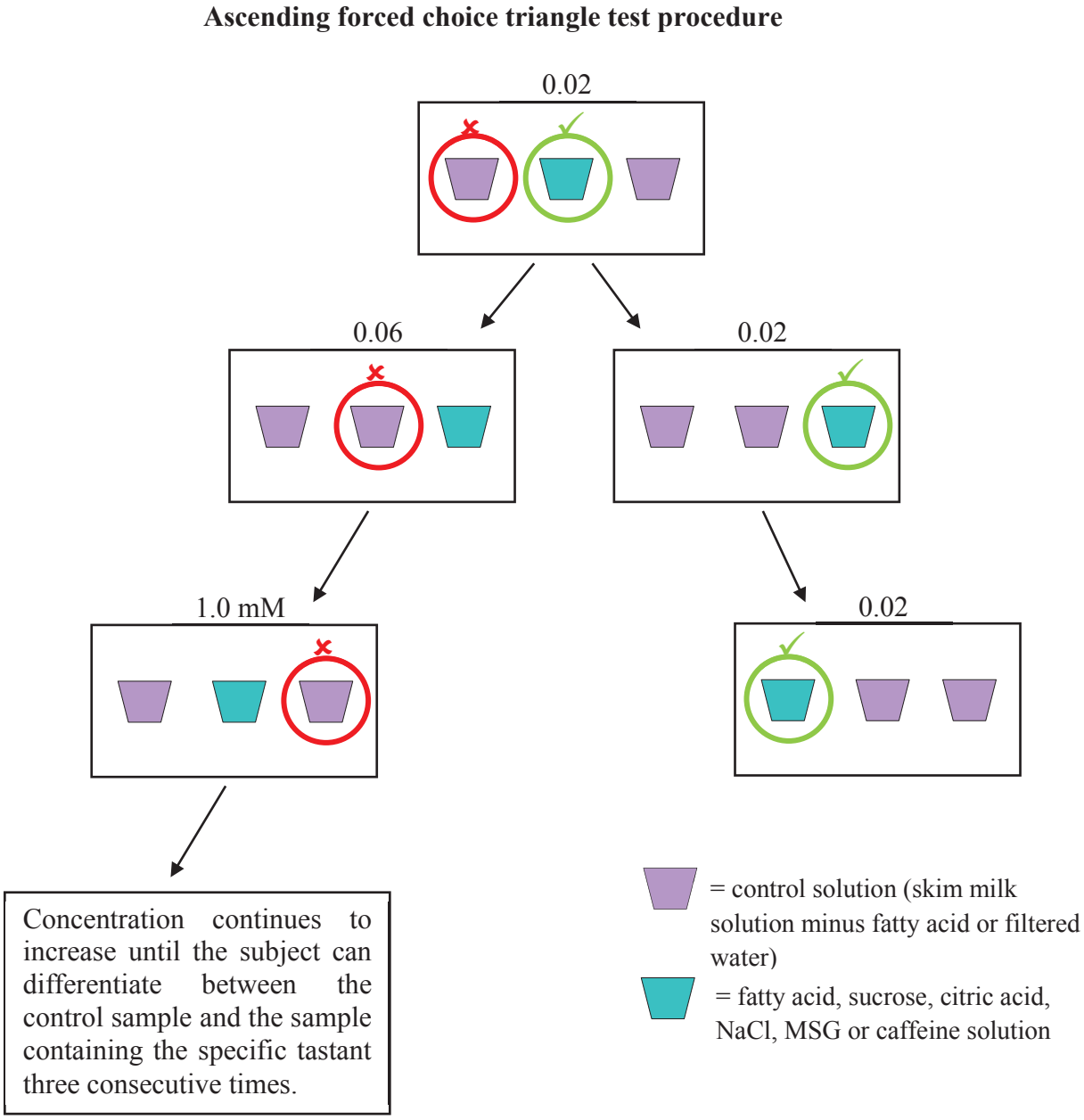


Figure 3.1: A flow diagram of the ascending forced choice triangle test procedure. Subjects are given one set of samples which contains two control samples and one sample containing a set concentration of the tastant (in this example 0.02 mM). Subjects then taste each sample and pick the sample which they think is the odd one

out. If the subject correctly identifies the odd sample, they are given a second tray at the same concentration and if they correctly identify the odd sample again, they are given a third tray at the same concentration. If they again correctly identify the odd sample, this concentration is deemed their oral detection threshold (in this case 0.02 mM). However, if the subject does not correctly identify the odd sample, they are given a second tray with the next highest concentration of tastant (0.06 mM) and again if they identify the incorrect sample the concentration increases. This continues until the subject can identify the odd sample correctly three consecutive times.

3.3.4 Primary taste qualities – materials and methods

To further investigate taste sensitivity to the five primary taste qualities, sample solutions for sweet (sucrose), sour (citric acid), salty (sodium chloride (NaCl)), bitter (caffeine) and umami (monosodium glutamate (MSG)) were prepared for study one and three. Solution concentrations for sucrose, citric acid, NaCl, caffeine and MSG were prepared in accordance with the International Organisation for Standardisation (ISO), ISO 3972:1991- Sensory analysis- Methodology- Method of investigating sensitivity of taste **(Table 3.1)**. For this, stock solutions of each tastant were made using 24 g sucrose (CSR White Sugar, Mackay, Queensland, Australia), 1.2 g citric acid anhydrous (Swiftco Ltd, Mulgrave, Victoria, Australia), 4 g NaCl (SAXA table salt, Cerebos Foods, Seven Hills, New South Wales, Australia), 0.54 g caffeine (SAFC Supply Solutions, St Louis, Missouri, USA) and 2 g MSG (Foodstuffs Co. Ltd, Lay Brothers, Dandenong, Victoria, Australia) and combining it with 1 L of filtered water.

Table 3.1: Specification and preparation of test solutions

Chemical	Concentrations (mM)							
Sucrose	35.05	21.03	12.62	7.56	4.55	2.75	1.61	0.99
Citric acid	3.12	2.50	1.98	1.61	1.30	1.04	0.83	0.68
NaCl	34.22	23.96	16.77	11.81	8.21	5.82	4.11	2.74
Caffeine	1.39	1.13	0.88	0.72	0.57	0.46	0.36	0.31
MSG	5.91	4.13	2.89	2.01	1.42	1.01	0.71	0.47

NaCl, sodium chloride; MSG, monosodium glutamate

3.3.5 Primary taste qualities – threshold measurement

Detection thresholds for sucrose, citric acid, NaCl, MSG and caffeine were determined using ascending forced choice triangle tests, using the same methodology as oral fatty acid detection thresholds. Filtered water was used as the control. All testing was performed under red lighting and nose clips were worn at all times.

3.3.6 6-*n*-propylthiouracil (PROP) sensitivity – materials and methods

The chemical compound 6-*n*-propylthiouracyl (PROP) is often used in taste research as a marker of global oral sensitivity. For example, an individual who experiences extreme bitterness from PROP will be classified as a 'supertaster', while another individual who experiences no bitterness from PROP will be classified as a 'nontaster' (Bartoshuk, Duffy, & Miller, 1994). The relevance to oral fatty acid sensitivity has not been determined; however some previous studies have suggested PROP tasters are more sensitive to orally delivered fats (Nasser et al., 2001). A 50

mmol/L PROP solution was prepared by dissolving 8.5 g of the PROP powder in boiling water (100ºC) on a stirring hotplate. Filter paper discs were cut to 1.5 cm in diameter and were dipped in the PROP solution for 30 seconds and then removed. Excess PROP solution was lightly shaken off and the discs were then laid out on an oven tray and placed in an oven heated at a temperature of 120ºC until dry (approximately 30 minutes). Dried samples were then cooled to room temperature, individually wrapped and stored in a plastic air-tight container at room temperature. The amount of PROP per filter paper has previously been measured as 0.28 mg/ filter paper (Zhao, Kirkmeyer, & Tepper, 2003).

3.3.7 PROP sensitivity – measurement

Subjects were presented with a piece of filter paper which contained the PROP solution and asked to place it on the centre of their tongue until it was soaked with saliva. Following this, subjects removed the paper from their tongue and were asked to rate the perceived intensity of the bitterness of the PROP using a general Labelled Magnitude Scale (gLMS). A gLMS is a scale which is used to not only rate the intensity of taste, but also all other senses, for example, pain, chemesthesis *etc.* It is the current gold standard for taste intensity rating and in this case it was used to rate the intensity of the bitterness of PROP. The gLMS has descriptors placed at varying positions which range from 'strongest imaginable' to 'barely detectable'. Considering this scale was 100 units, descriptors were placed at the following points; 0; neutral; 1.4; barely detectable; 6; weak; 17; moderate; 35; strong; 54; very strong and 100; strongest imaginable **(Appendix C)**. Subjects were briefly trained in the use of this scale and told that 'strongest imaginable' sensation was the pain caused if

a dentist drilled a hole in your tooth without any anaesthetic, and 'barely detectable' was tastes like paper.

3.3.8 Fat ranking task – materials and methods

An individual's ability to detect fatty acids in food may have no influence on their ability to detect a concentration of fat in foods; therefore a ranking task was developed to assess the subject's ability to identify fats within a common food (custard) (Stewart, et al., 2010; Stewart, Newman, et al., 2011). For this task, subjects were provided with custard made with (0%, 2%, 6% & 10%) canola oil. Custard was made from 20 g custard powder (Foster Clark's, Cerebos Foods, Seven Hills, New South Wales, Australia), 12 g sugar (CSR, Yarraville, Victoria, Australia) and 500 ml skim milk (Devondale, Cobram, Victoria, Australia). The ingredients were combined and heated in a conventional microwave oven until thickened. The custard was then removed from the microwave and set aside to cool to room temperature. The cooled custard was separated into four batches (100 g/ batch) and 2 ml, 6 ml or 10 ml of canola oil (You'll Love Coles, Coles, Springvale, Victoria, Australia) was added to three of the batches to create fat contents of 2%, 6% and 10% oil, respectively. No oil was added to one custard batch to create the 0% sample. All batches of the custard from 0% to 10% were sensory matched to contain 10% 'oil', which was added to the 0%, 2%, and 6% batches as paraffin, for example, the custard made with 10% oil, had 0 ml of paraffin added, for the 6%, 4 ml was added, for the 2%, 8 ml was added and for the 0%, 10 ml of paraffin was added.

3.3.9 Fat ranking task – measurements

Subjects were presented with the four 20 g custard samples (0%, 2%, 6% and 10%) in a randomised order and asked to rank them according to perceived fattiness. Subjects were instructed to taste each of the custard samples, starting from the left and moving through to the right. Subjects were advised to focus on the taste of each sample before swallowing it. Subjects were also told that they could go back and forth between the samples several times to determine the rank order. Custard samples were labelled with random 3-digit blinding codes. Subjects were asked to complete a specialised answer sheet in which they filled out the blinding codes according to the perceived fattiness of the samples, ranging from 1 = 'least fatty' to 4 = 'most fatty' **(Appendix D)**. All subjects received a score out of five for this task. The scores were calculated according to the combinations described in previous research (Stewart & Keast, 2012) **(Table 3.2)**. The chance of subjects guessing the correct order of custard is 2%. This task was completed on the same day as the threshold testing, but was always completed after the threshold testing.

Table 3.2: Scores for the fat ranking task

Combination	Score
0%, 2%, 6%, 10%	5
2%, 0%, 6%, 10%	4
0%, 2%, 10%, 6%	3
0%, 6%, 2%, 10%	2
10%, 2%, 6%, 0%	2
0%, 6%, 10%, 2%	1
6%, 0%, 2%, 10%	1
2%, 10%, 6%, 0%	1
10%, 2%, 0%, 6%	1
6%, 10%, 2%, 0%	0
10%, 6%, 0%, 2%	0
10%, 6%, 2%, 0%	0

Subjects received a score out of five for this task. Scores were based on the order in which the custards were ranked. All the subjects who placed the lowest sample (0%) next to the highest sample (10%) were scored zero for the task.

3.3.10 Hedonic test – materials and methods

During studies two and three, subjects were required to rate their liking of three sets of regular-fat (RF) and low-fat (LF) foods. The foods included cream cheese (RF: Philadelphia Spreadable Cream Cheese Original; LF: Philadelphia Spreadable Cream Cheese Extra Light, Kraft Foods Limited, South Wharf, Victoria, Australia), vanilla yoghurt (RF: Yoplait Creamy Original Vanilla yoghurt; LF: Yoplait Creamy Lite Vanilla yoghurt, National Foods, Docklands, Victoria, Australia) and chocolate

mousse (RF: Nestle Chocolate Mousse; LF: Nestle Diet Chocolate Mousse, Nestle, Fonterra Brands, Auckland, New Zealand). Twenty gram samples of each food, both regular-fat and low-fat, were presented to subjects during testing. Cream cheese, yoghurt and mousse were served at 4°C as they were stored under refrigeration. Tests were conducted without nose clips and after threshold tests and the fat ranking task.

3.3.11 Hedonic tests – measurements

Subjects were informed that they were to taste and rate their liking of the three sets of foods, however they were not informed that the samples differed in fat content. Subjects were given one set of samples at a time which were labelled with random 3-digit blinding codes. Subjects were asked to rate their liking of each sample using a 9-point hedonic scale **(Appendix E)**, which ranged from 1 = 'dislike extremely' to 9 = 'like extremely'.

3.4 Energy intake and habitual diet

3.4.1 Food records

Procedure

One-day food records in household measures were used to establish habitual diet and nutrient intake of each subject **(Appendix F)**. For studies one, two and three, which involved dietary information collection, subjects were thoroughly explained and shown how to complete a food record. Subjects were asked to complete the one-day food record in household measures in which they reported all foods and drinks consumed. Subjects were asked to, where possible, weigh the foods they consumed

(subjects used their own scales), or use standard metric measuring cups, or common serving sizes, for example one cup of cereal. They were also asked to report the brand of food consumed, type of food (*e.g.,* white or wholemeal bread), whether fat was added (*e.g.,* butter) and the method of cooking (*e.g.,* frying, steaming, baking). If food was consumed from a recipe, subjects were asked to include the recipe and state how much of the recipe was consumed (*e.g.,* quarter, whole).

During studies one and two, subjects completed a food record within the week prior to sensory testing. During study three, subjects completed five food records (once a week); one at baseline and one during week two, three, four and six.

Analysis

The one-day food records were analysed using FoodWorks 2009 (Xyris software, Highgate Hill, Queensland, Australia) using the AUSNUT 2007 database for foods, brands and supplements. From these data, the mean energy intake (kJ) and macronutrient distribution (percentage energy from fat, protein and carbohydrate, and grams of fat, protein and carbohydrate) and the type of fat (grams and percentage of monounsaturated, polyunsaturated or saturated), salt (mg), and alcohol (g) was determined.

3.4.2 Food Frequency Questionnaire

Procedure

During study three, subjects were required to complete a food frequency questionnaire, which was adapted from the 1995 National Nutrition Survey

(Australian Bureau of Statistics, 1995) **(Appendix G)**. This was used to evaluate the types of foods that were consumed during the month prior to the commencement of each study (Australian Bureau of Statistics, 1995). In total, ten categories of food were assessed including dairy products; breads and cereals; meat, fish and eggs; other offal; sweets, baked goods and snacks; dressings; non-dairy beverages; vegetables; fruits; and vitamin and mineral supplements and specifically 120 food items were assessed in section one of the questionnaire. The frequency they could be consumed ranged from 'never or less than once a month' to 'six or more times per day'. In section two, questions identifying dietary behaviours were asked including 'what type of milk do you usually consume?', 'how often is the meat you eat trimmed of fat either before or after cooking?', 'how often do you add salt to your food after it is cooked?' or 'how many serves or fruits or vegetables do you usually eat each day?' *etc.*

Analysis

Each question of section one of the food frequency questionnaire inquired about the frequency of consumption of specific types of foods, for example, 'white bread, toast or rolls'. Each frequency category was converted into a daily equivalent value, for example, 'never, or less than once a month' = 0.02, 'one to three times per month' = 0.07, 'once per week' = 0.1, 'two to four times per week' = 0.4, 'five to six times per week' = 0.8, 'once per day' = 1.0, 'two to three times per day' = 2.5, 'four to five times per day' = 4.5 and 'six plus times per day' = 6. The foods were then categorised according to specific food groups, for example, red meat; poultry; processed meat; organ meat; fish; seafood; refined grains; whole grains; eggs; high-

fat dairy; low-fat dairy; soy; liquor spirits; wine; beer; hot drinks; fruit; leafy vegetables; cruciferous vegetables; other vegetables; tomatoes; peas and legumes; nuts; potatoes; high-fat take away foods; fried foods; snacks; desserts and biscuits; chocolate and sweets; sugar sweetened beverages; low-energy beverages; condiments; and salad dressing. For section two, each answer option was coded ranging from 1 to 6, for example, for the question what type of milk do you consume, 1 = 'whole milk', 2 = 'low/ reduced fat milk', 3 = 'skim milk', 4 = 'evaporated or sweetened condensed milk', 5 = 'none of the above' and 6 = 'don't know'. Each subject received a numerical score for each question.

3.5 Fat pre-load meals

For study two, subjects were required to consume three different breakfast frittatas (high-fat, low-fat and macronutrient balanced) on three separate occasions. Subjects were provided with and asked to consume a standardised dinner (Lean Cuisine Beef Lasagne, Simplot, Mentone, Victoria, Australia) on the evening prior to each testing session and were asked not to consume any other foods (water was allowed) until attending the sensory laboratory the next morning for breakfast. A breakfast frittata was chosen as the breakfast food as it could be easily manipulated to contain different percentages of macronutrients and all frittatas were made in the Deakin University Sensory Laboratory by the researcher and cooked freshly each day. On the morning of testing, subjects were given one of three different frittatas which varied in macronutrient content (high-fat: 60% fat, 20% protein, 20% carbohydrate; low-fat: 20% fat, 40% protein, 40% carbohydrate; macronutrient balanced: 33% fat, 33% protein and 33% carbohydrate) **(Appendix H)**. The breakfasts were matched

for energy and the kilojoule content for each frittata was 1500 kJ. To compensate for variation in the volume of each breakfast, breakfasts were equated with a measured amount of drinking water. Subjects were advised that they were to consume the whole breakfast. Once the frittata had been completely finished by the subject, they were asked to come back to the laboratory twice to measure their oral fatty acid sensitivity; once one hour after completion of the breakfast and the other one hour after completion of the first detection threshold test. Between each testing session subjects were asked to refrain from eating, drinking or chewing gum.

3.6 Dietary counselling

For study three, subjects participated in a six week randomised dietary intervention study. Subjects were randomised to one of two diets; a low-fat diet (25% total energy from fat) or a portion control diet (33% total energy from fat, reduction in energy intake by 25%) for a six-week period.

3.6.1 Low-fat diet

This 25% fat diet was based on the OZDASH diet and was designed to reduce the consumption of full fat dairy products, fatty red meats, baked products, high-fat spreads and overall kilojoule intake (Margerison et al., 2003). Dietary counselling was overseen by the coordinating dietitian (S.T.) and provided by trained research staff. Subjects were given a face-to-face counselling session at baseline, which took between 30-45 minutes and a booklet which contained all of the information needed to follow the diet, specifically how many portions of particular foods they should aim to eat and what constituted a portion **(Appendix I)**. One portion of dairy = milk

(200 ml), yoghurt (200 g) or cheese (40 g); one portion of fruit = 1 medium piece of fruit (100 g) or fruit juice (200 ml); one portion of vegetables = ½ cup cooked vegetables (50 g), potato (90 g), 1 cup leafy green vegetables; one portion of meat and fish = cooked meat or chicken (100 g), fish (80-100 g), one portion of fats and oils = 1 teaspoon monounsaturated or polyunsaturated oils, 1 teaspoon of regular-fat margarine, 2 teaspoons reduced-fat margarine, 2 teaspoons avocado; and one portion of breads and cereals = 2 slices of bread, 1 cup cooked rice or pasta, 1 cup porridge or breakfast cereal, ½ cup muesli. Subjects were instructed to consume at least five portions of vegetables and two portions of fruit per day, as well as three portions of low-fat dairy per day, one to two portions of meat and fish per day, a maximum of three portions of fats per day and a maximum of five portions of breads and cereals per day. The booklet also contained a list of snack food ideas, tips on how to reduce kilojoule intake from day to day, as well as recommendations when dining outside of the home, a section on how to read food labels, and an example meal plan. To assist with compliance, subjects were given a low-fat margarine (Flora Ultra-Light, Unilever, Epping, New South Wales, Australia), four packets of low-fat crackers (Sakata Rice Snacks Australia, Laverton North, Victoria, Australia), and one packet of popcorn kernels (Popping Corn, Riviana Foods, Scoresby, Victoria, Australia) as alternatives to high-fat spreads and snack foods. Subjects were also contacted by phone on a weekly basis throughout the six-week period to answer any questions that may have arisen and to keep subjects motivated and accountable.

3.6.2 Portion control diet

The portion control diet was designed to decrease subjects' energy intake by 25%. This diet was based on the Australian government campaign, 'Swap it, don't stop it,' (2012). Subjects were instructed to reduce the consumption of their usual diet. Subjects from this group also had a one-on-one session with trained research staff, which took between 20-40 minutes, for dietary counselling to reduce their energy intake by 25%, for example, reducing the amount of food on their plate at dinner time, but keeping the same proportion of each food component. Subjects were also given a list of instructions to help reduce meals sizes and this provided the following four key concepts **(Appendix J)**; 1) use a smaller plate as this makes a little food seem a lot, 2) eat mindfully by taking time to chew your food properly and eat slowly (Smit, Kemsley, Tapp, & Henry, 2011), 3) avoid distractions such as watching television or reading as this can distract you from noticing when you are full, 4) follow the 80 percent rule which was to stop eating before you are completely full, which ties in with concept two ("Sizing up food portions," 2010). Subjects were also given some ideas on how to reduce meal size when eating out of the home, for example, order an appetiser or two as opposed to an appetiser and main meal. To aid in compliance, subjects were given a small, appetiser sized plate (23 cm) for all meals, as studies have found by using a smaller plate, subjects are more likely to eat less (Laddu et al., 2011; Pedersen, Kang, & Kline, 2007; "Sizing up food portions," 2010). Weekly calls were made to those following the portion control diet to answer any underlying questions and to keep subjects motivated.

3.7 Physical activity

Study three encouraged subjects to make healthy lifestyle changes, therefore all subjects were required to participate in a minimum of 30 minutes of moderate intensity physical activity on all or most days of the week of the intervention (Sims, Hill, Hunt, & Haralambous, 2010). Subjects were advised to work at a level where their heart rate was 60-70% of their maximum heart rate. Subjects were provided with information on how to calculate their maximum heart rate (220 – age (years)) and the researcher calculated this for each subject during their dietary counselling session. This level was considered moderate intensity and subjects were advised to exercise at this level for the full 30 minutes. The amount of exercise completed was measured at baseline and week six using the Community Healthy Activities Model Program for Seniors (CHAMPS) questionnaire **(Appendix K)** (Stewart et al., 2001). The questionnaire evaluated 41 different types of exercise and questions included, 'how many times on average in the past week did you do moderate intensity strength training?' or 'how many times on average in the past week did you go for a walk for exercise?' Subjects were to complete the number of times they completed each activity per week and the number of hours spent doing this activity per week. The number of hours completed per week were categorised and coded so that 1 = 'less than one hour per week', 2 = 'one to two and a half hours per week', 3 = 'three to four and a half hours per week', 4 = 'five to six and a half hours per week', 5 = 'seven to eight and a half hours per week' and 6 = 'nine hours or more per week'. The average number of times per week each activity was completed and the average number of hours of exercise per week were calculated for the intervention period.

3.8 Tongue papillae

3.8.1 Tongue photography

Subject's tongues were photographed in study three to determine the number of tongue papillae, specifically fungiform papillae. Before the photograph was taken, subjects were asked to rinse their mouths thoroughly with deionised water. They were then asked to sit at the photography bench with their elbows on the bench and their hands held together in a 'V' formation supporting their chin and keeping their head still **(Figure 3.2)**. Subjects were then asked to protrude their tongue and use their lips to keep it steady in order to produce a clear photograph for accurate counting of fungiform papillae. Once subjects were set up in this position, the camera (Nikon D90 Digital Camera, Nikon, AF-S Micro Nikkor 105 mm lense, Nikon Australia Pty. Ltd, Rhodes, New South Wales, Australia) was set up on the tripod (SLIK Corporation, SBH100DQ tripod, Hidaka City, Saitama, Japan) and precisely lined up with the participants tongue. The tongue was then dried using a 50 mm x 20 mm strip of filter paper. Using a cotton bud (Swisspers, McPhersons Consumer Products, Kingsgrove, New South Wales, Australia), diluted blue food dye (Queen Fine Foods, Alderley, Queensland, Australia) (1 x drop dye : 20 x drop deionised water) was then applied to the left of the midline of the tongue on the tip, as this area shows a high correlation to the total number of papillae on the tongue (Shahbake, Hutchinson, Laing, & Jinks, 2005). The tongue was then dried with a strip of filter paper again to remove excess dye. A square piece of filter paper (10 mm x 10 mm) with a 6 mm hole in the centre and labelled with the subject's identification code, was then place on the tip of the tongue over the area that had been dyed with the blue food dye. Three macro photographs were then taken of the

subject's tongue **(Figure 3.3)**. Subjects were required to have their tongue protruded throughout the whole procedure, from dying the tongue until the final three photographs had been taken.

Figure 3.2: The position of the subject and the distance between the tongue and camera required for the tongue papillae photograph

3.8.2 Counting of tongue papillae

The photos were loaded onto the computer and were analysed using the Adobe Photoshop version CS5.1 (Adobe Systems Inc., San Jose, CA, USA). Each subject's photo was coded with the subject's identification code in order to avoid any bias when collating results. Fungiform papillae are generally mushroom shaped, elevated structures, however some can be flat with little elevation or double papillae (Shahbake, et al., 2005). The fungiform papillae were identified as the structures that were stained in a very light shade of blue. The dark shades were the filiform papillae

(Shahbake, et al., 2005). The counting tool in Adobe Photoshop was used to count the number of fungiform papillae to ensure that each papilla was only counted once **(Figure 3.4)**. When papillae were hard to distinguish or difficult to confirm, the zoom function in Adobe Photoshop was used to magnify the image.

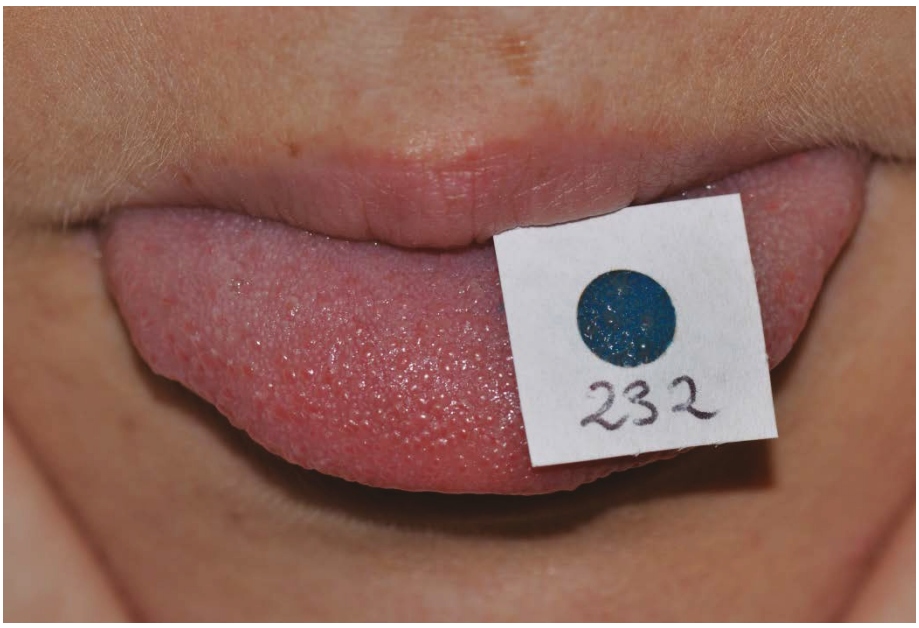


Figure 3.3: A photograph of the tongue papillae

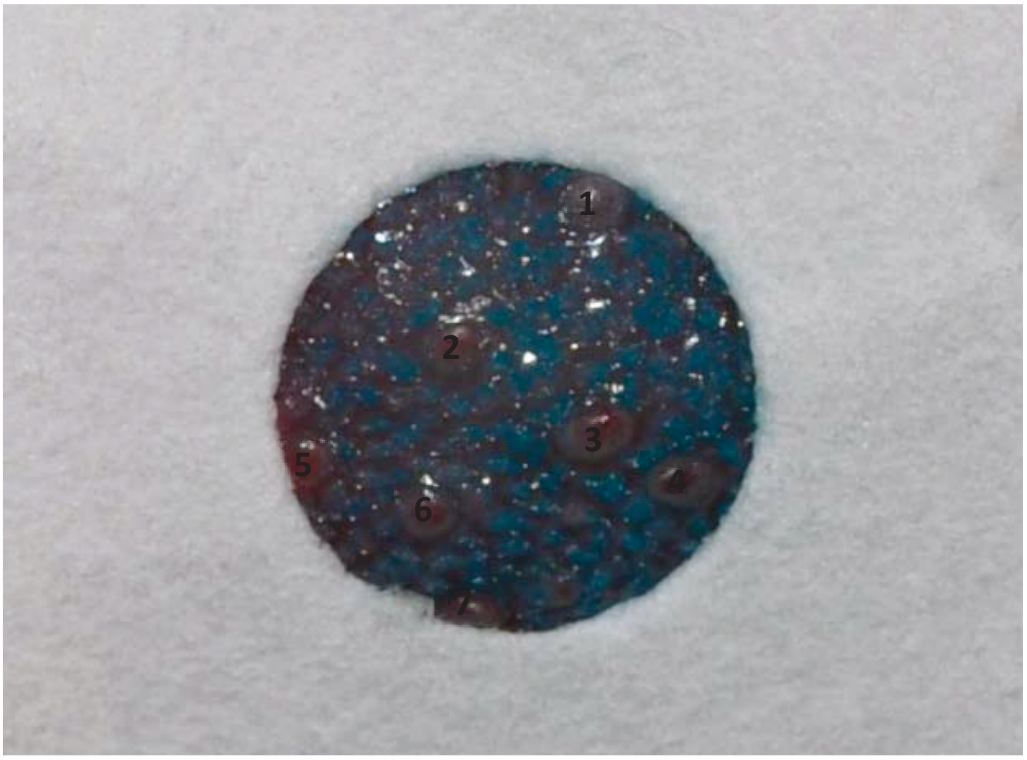


Figure 3.4: Magnification and identification of fungiform papillae from photographs

3.9 Statistical analysis

3.9.1 Study 1 – The test-retest reliability of oral fatty acid detection thresholds

All data were analysed using SPSS version 21 (SPSS Inc., Chicago, Illanois, USA). Paired samples t-tests were used to determine if there were any differences between detection thresholds for fatty acids between sessions on the same day and across days. Intra-class correlation (ICC) analysis was performed to detect associations between fatty acid detection thresholds within and between testing days for each fatty acid (C18:1, C18:2, C12:0) and prototypical taste stimuli. ICC correlations > 0.7 were classified as a strong correlation, whilst ≤ 0.7 was classified as moderate to low in strength. Pearson's correlations were used to detect associations between fatty acid detection thresholds between C18:1, C18:2 and C12:0 as well as associations

between detection thresholds for fatty acids and total fat, saturated fat, polyunsaturated fat and monounsaturated fat intakes, carbohydrate, protein intake and energy intake. A correlation > 0.7 was classified as strong, whilst ≤ 0.7 was classified as moderate to low (Pallant, 2011). Fatty acid thresholds were calculated as the mean of all six values when assessing correlations with macronutrient and energy intakes. Significance was accepted at $P < 0.05$.

3.9.2 Study 2 – The influence of a high-fat meal immediately prior to oral fatty acid detection threshold testing

Differences in oral fatty acid sensitivity after each breakfast were compared using one-way repeated measures analysis of variance (ANOVA). Within day differences in oral fatty acid sensitivity were determined using paired samples t-tests. Reliability analysis was conducted using ICC to detect associations between C18:1 detection thresholds within and between testing days. ICC > 0.7 were classified as strong correlations, whilst ≤ 0.7 was classified as moderate to low in strength (Pallant, 2011). One-way repeated measures ANOVA was also used to detect differences between preferences for regular- and low-fat foods after each breakfast and Friedman tests were used to detect differences between fat ranking scores after each breakfast. Independent samples t-tests were used to detect differences in oral fatty acid sensitivity, gender and healthy weight category as well as differences in energy and macronutrient intake between lean and obese subjects. Pearson's correlations were used to detect associations between C18:1 detection thresholds and total energy, fat, carbohydrate and protein intake, as well as percentage energy from fat,

carbohydrates and proteins. A correlation > 0.7 was classified as strong, whilst ≤ 0.7 was classified as moderate to weak. Significance was accepted as $P < 0.05$.

3.9.3 Study 3 – The effect of dietary fat consumption and weight loss on oral fatty acid sensitivity

Repeated-measures ANOVA was used to analyse changes in C18:1, sucrose and NaCl oral detection thresholds, hedonic ratings for regular-fat and low-fat foods, anthropometric measurements, tongue papillae numbers and dietary intake from baseline to week six with time-point as within-subject and dietary intervention (low-fat or portion control) as between-subject factors. To detect differences in fat ranking scores from baseline to week six, Wilcoxon Signed-Rank test was used and between group analysis was conducted using Mann-Whitney U test. Significance was accepted at $P < 0.05$. Paired t-tests with Bonferroni correction were used to establish differences in answers to dietary questionnaires from baseline to week six. Significance was accepted as $P < 0.05$.

CHAPTER FOUR

Study 1

The test-retest reliability of oral fatty acid detection thresholds

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4.1 Introduction

It is widely acknowledged that humans perceive five taste qualities which include sweet, sour, salty, bitter and umami. The taste system most likely evolved so that animals chose foods that were appropriate for their body's needs and it is for this reason that the sense of taste can also be referred to as an oral nutrient detection system; as the oral cavity contains specific receptors for certain nutrients, for example, sugars (carbohydrate, sweet) and amino acids (protein, umami) (Bachmanov & Beauchamp, 2007). From an evolutionary point of view, this nutrient detection system was used to detect the nutritional or toxic quality of foods whereby sweet, salty and umami are associated with positive appetite responses to increase the chance of consumption of essential nutrients, whereas, excessive bitter and sour elicit an aversive response to reduce the consumption of these foods (Bachmanov & Beauchamp, 2007; Breslin, 2013). However, humans seem to be able to tolerate low levels of bitterness and often learn to enjoy the taste of mildly bitter foods when paired with more liked attributes or positive metabolic and pharmacological outcomes such as in chocolate, coffee or wine (Breslin, 2013). It seems logical that as well as sensing carbohydrates and proteins we may also have a mechanism for sensing fat as it is an essential macronutrient for humans. However, it is unknown

whether this detection results in an appetitive or aversive response due to the acidic nature of fatty acids. A dose response effect for taste perception is common among tastants, for example low levels of a salt provide little or no perception for humans, therefore neither an appetitive nor aversive response, moderate levels of salt provide an appetitive response and high levels produce an aversive response (Breslin, 2013). Emerging evidence now indicates the existence of an oral fat specific detection system which is activated by the breakdown products of fat, fatty acids, to convey the presence of fat in foods.

A taste quality is perceived when the concentration in the oral cavity reaches a particular level that activates a specific receptor, which in turn elicits a perception (Keast & Roper, 2007). For example, an aqueous solution may contain a compound such as sodium chloride (NaCl), but at a concentration that cannot be detected. As the concentration of the NaCl increases, the aqueous solution can be discriminated from water and a detection threshold is reached (Keast & Roper, 2007). As the concentration increases further, the recognition threshold will be reached whereby the quality (salty) can be identified (Keast & Roper, 2007). What constitutes a taste quality has been debated for many years (Delwiche, 1996). Arguably it must have four criteria; 1) a defined class of effective stimuli, 2) a unique transduction mechanism as part of taste cells, 3) peripheral taste mechanisms conveyed by gustatory nerves and 4) be distinguishable from other taste stimuli (Mattes, 2011a). For example, sweet taste in humans, 1) sucrose and other sugars are the stimuli in which humans can detect in the oral cavity, 2) sugars are transduced via G-protein coupled receptors (T1R2-T1R3 dimer) on tongue papillae, 3) the signal from the

receptor is carried by both the chorda tympani nerve and glossopharyngeal nerve to the brain and 4) sweet taste can be perceptually distinguished from the other four taste qualities (Gilbertson, et al., 2000). In addition, it is thought that fats can be perceived across multiple modalities including olfaction, vision, texture, audition and chemesthesis (Tucker & Mattes, 2012). Emerging evidence also indicates the components of fats, fatty acids, can be orally identified at detection threshold levels in humans when non-taste cues are minimised (Chale-Rush, et al., 2007a; Mattes, 2009d; Stewart, et al., 2010; Stewart, Newman, et al., 2011). However, although the results from studies investigating oral fatty acid detection thresholds seem to imitate results found for the primary tastes, challenges arise due to the unique nature of the fatty acid stimuli, for example, there is no established lexicon for fatty acid detection making testing difficult for participants who have not experienced the sensation before (Running, et al., 2013).

Recently, studies have identified fatty acid receptors cluster of differentiation 36 (CD36) and G-protein coupled receptor (GPCR120, GPCR40) on taste cells in humans for the identification of fatty acids in the oral cavity (Ichimura, et al., 2012; Simons, et al., 2010). Results from a study involving mice found that when the *CD36* receptor on taste cells was inactivated, a reduction in preference for long chain fatty acid enriched solutions and solid foods was seen (Laugerette, et al., 2005). Similarly, GPCR120 and GPCR40 knock-out mice showed an attenuated preference for oleic acid (C18:1) and linoleic acid (C18:2), suggesting that these two GPCRs contribute to preference for fatty acids through the gustatory system (Cartoni, et al., 2010). It is important to note that these receptors are influential over the transduction

mechanisms involved in the detection of fatty acids within the oral cavity and the differences seen in preference may be due to differences in transduction mechanisms and receptor expression.

Recent evidence in humans now suggests that during mastication of a high-fat food, a sufficient concentration of non-esterified fatty acids (NEFA) are released in the oral cavity to initiate gustatory signalling (Kulkarni & Mattes, 2013). Previous research has also reported that humans can detect a taste quality at a faster rate than they can identify the intensity of the quality (Halpern 1983). However, the aversive quality of fatty acids is only apparent at concentrations when an actual quality is perceived and the quality is most likely associated with aroma and oro-nasal irritation, rather than taste. We speculate that an appetitive response associated with fatty acids come via subconscious feedback mechanisms within both the oral cavity and the gastrointestinal (GI) tract. This may be a coordinated response whereby the oral cavity sends signals to the brain and which are associated with changes in the GI tract and thus a satiety cascade (Newman, et al., 2013). Furthermore, in previous research and this study, oral thresholds for C18:1, C18:2 and lauric acid (C12:0) did not correlate with thresholds for the other prototypical tastants, which suggests that oral fatty acid transduction mechanisms are independent from oral mechanisms for other tastants (Mattes, 2009d; Stewart, et al., 2010). These findings suggest the existence of an independent oral transduction system for fatty acids.

Numerous animal and human studies have found that individuals can have varying taste sensitivities to fatty acids and that their taste sensitivity to fatty acids may be

associated with the dietary consumption of fat. Gilbertson (1998) investigated this relationship in rats, whereby it was found that fat insensitive (Osborne-Mendel (O-M)) rats showed a preference for high-fat chow and rapidly gained weight when exposed to a high-fat diet, whereas fat sensitive (S5B/PI) rats preferred high-carbohydrate chow and resisted weight gain when exposed to a high-fat diet (Gilbertson, et al., 1998). In addition, a recent study in humans has reported an association between C18:1 detection thresholds and an individual's dietary fat intake and body mass index (BMI), whereby those who were insensitive to C18:1 consumed more dietary fat and had higher BMIs than those who were sensitive (Stewart, et al., 2010). A separate study confirmed the previous findings and also found that those who were insensitive to C18:1 consumed more high-fat dairy products as well as high-fat spreads and fatty red meats, which are some of the major contributors to fat intake in the Western diet (Stewart, Newman, et al., 2011). Links between the GI tract, BMI and oral fatty acid sensitivity have also been made, whereby obese individuals were less orally sensitive to fatty acids and consumed more energy at a buffet meal post intra-duodenal C18:1 infusion than lean individuals (Stewart, Seimon, et al., 2011). However, not all studies have found links between oral fatty acid sensitivity and BMI (Kamphuis, Saris, et al., 2003; Mattes, 2009d, 2011b; Stewart & Keast, 2012), with some authors hypothesising that this may be due to subjects being classified as hyper- and hyposensitive (Running, et al., 2013). Research has also demonstrated the elasticity of oral fatty acid thresholds whereby an individual's oral sensitivity to C18:1 could be either increased or decreased depending on the amount of dietary fat consumed (Stewart & Keast, 2012). Conversely, other studies investigating possible links between oral fatty acid

sensitivity, fat intake and chronic disease have failed to find any links (Kamphuis, Saris, et al., 2003; Mattes, 2009d, 2011b; Stewart & Keast, 2012). Due to the potential plasticity/ elasticity of oral fatty acid sensitivity when the environment is altered, an essential and to date missing step in furthering evidence for oral fatty acid detection is to establish reliability and reproducibility of the oral fatty acid threshold method.

Research dating back to the 1950s has investigated the reproducibility of the primary tastes over numerous sessions with much debate over the stability of measurement. Cicerale et al. (2009) determined that the reliability of sucrose intensity over six sessions and found that each session was highly correlated ($r = 0.98$) (Cicerale, et al., 2009). The test-retest reliability of sucrose, citric acid, NaCl and caffeine were examined using tasting tablets and the three-drop method with high reproducibility being found for both methods (taste tablet: $r = 0.69$; three-drop method: $r = 0.71$) (Ahne, et al., 2000). Similar findings were also reported by Mueller et al. (2011) with high between session correlations found ($r = 0.77$). In contrast, Pangborn et al. (1959) examined the reliability of sucrose, NaCl, citric acid and caffeine using the 'choice method' and reported that in one session, young subjects (below the age of 27 years) could be the most sensitive in the cohort to a tastant, but in the next session they could be the least sensitive to the same tastant (Pangborn, 1959). Stevens et al. (1995) investigated age and reliability of taste thresholds for sucrose using a forced choice version of up-down tracking and reproducibility was very low (Stevens, et al., 1995). These studies all used different methodology which may be the cause of such variation in findings. Therefore, this study investigated the test-retest reliability of

oral fatty acid detection thresholds, as well as those for sucrose, citric acid, NaCl, caffeine and monosodium glutamate (MSG) using triangle tests with ascending forced choice triangle test methodology.

4.2 Aims and hypotheses

4.2.1 Aim

To investigate the reliability and reproducibility of oral fatty acid detection thresholds for saturated, monounsaturated and polyunsaturated fatty acids.

4.2.2 Hypotheses

There will be large inter-individual variability in oral fatty acid detection thresholds and there will be minimal intra-individual variation in oral fatty acid thresholds, therefore high test-retest reliability.

4.3 Subjects, materials and methods

This study was approved by the Deakin University Human Research Ethics Committee and was initiated on September 1, 2010. All testing involving human subjects was in compliance with the principles laid down in the Declaration of Helsinki and all subjects provided informed, written consent prior to participation.

4.3.1 Subjects

Subjects were recruited from Deakin University and surrounding suburbs. All subjects were over 18 years of age, were non-smokers and were in good health at the time of investigation. A total of 20 subjects gave written consent to complete the study and complete data were obtained from 17 subjects.

4.3.2 Study outline

Subjects were required to attend 30 laboratory sessions; three sessions per day, for ten days (not consecutive) to determine oral detection thresholds for C18:1, C18:2 and C12:0. Each stimulus was evaluated on six separate occasions, over two days. The three sessions on each day were at least two hours apart and each subject came at the same time on each testing day. Taste thresholds for the five prototypical tastes were performed using sucrose (sweet), citric acid (sour), NaCl (salty), caffeine (bitter) and MSG (umami). At the start of each testing day, subjects recorded their food consumption from that morning. At each session, subjects had their oral sensitivity assessed using ascending forced choice triangle tests, which has been used previously for this purpose (Stewart, et al., 2010). Each day, subjects were tested for a different taste quality. Height and weight of subjects were measured on day ten.

4.3.3 Oral fatty acid solutions

Food grade fatty acids (C18:1, C18:2 and C12:0) were added to long-life non-fat milk (Devondale, Cobram, Victoria, Australia) at varying concentrations (0.02, 0.06, 1, 1.4, 2, 2.8, 3.8, 5, 6.4, 8, 9.8 and 12 mM). As per the methodology outlined in **Chapter 3**, all samples were mixed with gum acacia, liquid paraffin and

ethylenediaminetetraacetic acid (EDTA). A stable emulsion was created by mixing the milk and gum together first and then this mixture was added to the paraffin and fatty acid mixture. Samples were homogenised at 12 000 rpm for 30 seconds/ 100 ml. All samples were freshly prepared each day of testing and C18:1 and C18:2 were served at room temperature, while C12:0 was heated to 50°C to ensure it was in a liquid state. Control samples were prepared in the same way, without the fatty acids.

4.3.4 Oral fatty acid detection thresholds

Oral fatty acid sensitivity was determined via detection thresholds, using ascending forced choice triangle tests **(Chapter 3, Figure 3.1)**, whereby subjects were presented with three samples, two control and one containing a certain concentration of fatty acid in an ascending order from the lowest concentration (0.02 mM) to the highest (12 mM) (Stewart, et al., 2010). Detection thresholds for each fatty acid were defined as the lowest concentration at which the subject correctly picked the odd sample out in three consecutive sample sets. All testing was performed under red lighting and nose clips were worn at all times.

4.3.5 Taste thresholds: sweet, sour, salty, bitter and umami

Solution concentrations for sucrose, citric acid, NaCl, caffeine and MSG were prepared in accordance with the International Organisation for Standardisation (ISO), ISO 3972:1991- Sensory analysis- Methodology- Method of investigating sensitivity of taste as detailed in **Chapter 3 (Table 3.1)**. Distilled water was used as the control sample in threshold determinations. All procedures were the same as those for oral fatty acid detection thresholds.

4.3.6 Food consumption

For each testing day, subjects recorded everything they consumed from the time they awoke in the morning **(Appendix F)**. Subjects were asked to estimate the amount of foods they consumed by using standard metric measuring cups, approximate weights or common serving sizes (outlined in **Chapter 3**). Food consumption was analysed using FoodWorks 2007 (Xyris software, Highgate Hill, Queensland, Australia) to ensure that subjects were consuming a typical diet. From this, the mean energy intake (kJ), macronutrient distribution (% energy from fat, protein and carbohydrate, and grams of fat, protein and carbohydrate) and the type of fat (grams and % of monounsaturated, polyunsaturated or saturated) were calculated.

4.3.7 Anthropometry

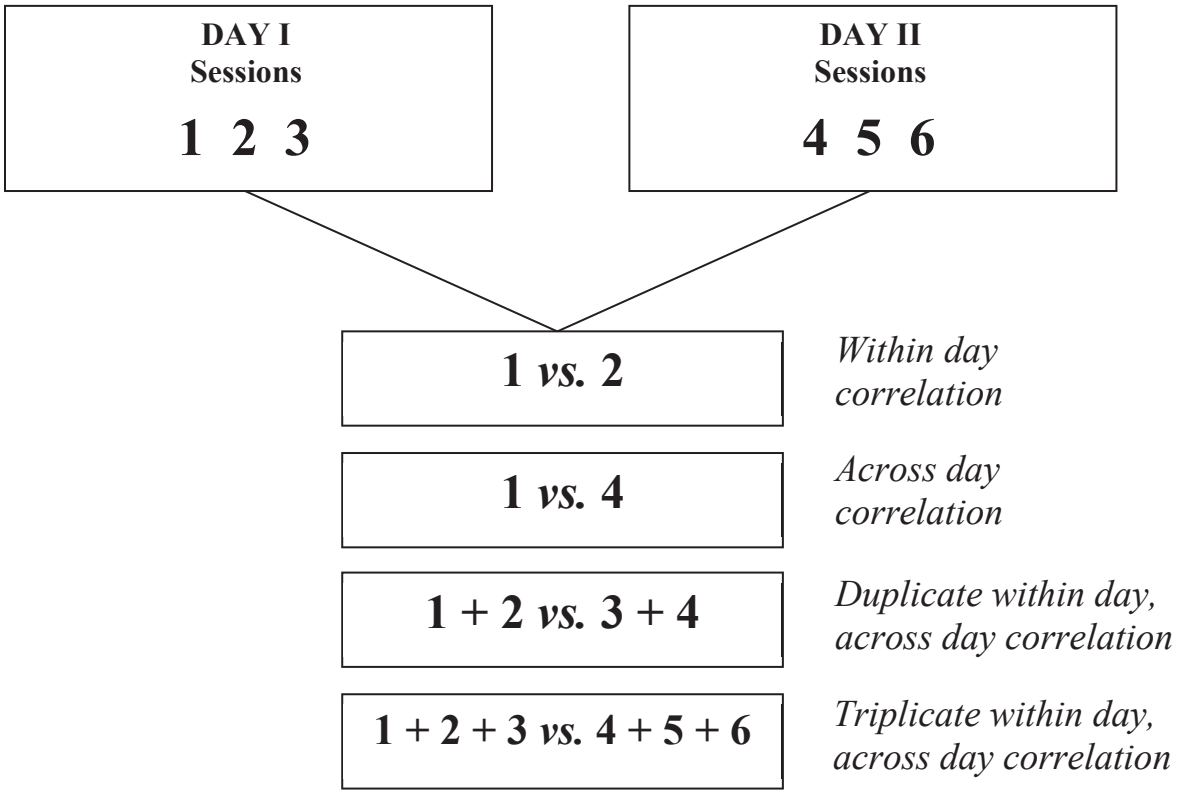
Weight and height were collected for all subjects without shoes and in light clothing using scales (Tanita Body Scan Composition Monitor Scales, Cloverdale, Western Australia, Australia) and a stadiometer (Seca, MedShop Australia, Fairfield, Victoria, Australia), respectively. BMI was calculated from these measurements (BMI = weight (kg)/ height (m^2) (Harris et al., 2008).

4.4 Statistical analysis

All data were analysed using SPSS version 21 (SPSS Inc., Chicago, Illanois, USA). Paired samples t-tests were used to determine if there were any differences between detection thresholds for fatty acids between sessions on the same day and across days. The data were analysed according to **Figure 4.1**. Intra-class correlation (ICC) analysis was performed to detect associations between fatty acid detection thresholds

within and between testing days for each fatty acid (C18:1, C18:2, C12:0) and prototypical taste stimuli. Pearson's correlations were used to detect associations between fatty acid detection thresholds between C18:1, C18:2 and C12:0. Pearson's correlations were also used to detect associations between detection thresholds for fatty acids, and total fat, saturated fat, polyunsaturated fat and monounsaturated fat intakes, carbohydrate and protein intake, as well as energy intake. All data are presented as means ± SEM.

Figure 4.1: The breakdown of sessions for each day and a description of how data analysis was conducted



4.5 Results

4.5.1 Subjects

Subjects (*n* = 17, 7 males: age 28.1 ± 2.3 years [range 22-33 years], BMI 22.1 ± 0.6 kg/m^2 [range 20.9-24.5 kg/m^2], 10 females: age 29 ± 1.8 years [range 21-45 years], BMI 23.4 ± 0.9 kg/m^2 [range 19.3-33.1 kg/m^2]) were recruited and two out of the total 17 participants were overweight/ obese (*n* = 2, 2 female, BMI 29.6 ± 0.8 kg/m^2). All subjects had measurable thresholds for the three fatty acids and the five taste qualities **(Table 4.1)**.

4.5.2 Oral fatty acid detection thresholds

C18:1, C18:2 and C12:0 detection threshold reliability

Oral detection thresholds were established by all subjects for C18:1, C18:2 and C12:0 (C18:1, 2.64 ± 0.7 mM [range 0.26-12 mM], C18:2, 1.41 ± 0.9 mM [range 0.04-4.7 mM], C12:0, 1.80 ± 0.4 mM [range 0.04-6.5 mM]) **(Figures 4.2 and 4.3)**. For each subject, there were no significant differences found between oral detection thresholds for C18:1, C18:2 and C12:0 for any sessions ($P = 0.257$, $P = 0.485$, $P = 0.411$, respectively) **(Figure 4.4)**. For C18:1, C18:2 and C12:0, strong correlations were found for within day testing sessions one and two. Strong correlations were also found for across day triplicate testing for all three fatty acids and for duplicate across day sessions for C18:2 only. Moderate strength correlations were found for duplicate testing across days both C18:1 and C12:0. Moderate to strong correlations for C18:1, C18:2 and C12:0 were found when comparing thresholds of one individual across the two days **(Table 4.1)**.

Table 4.1: Within day, across day, duplicate across day, triplicate across day and day within subject intra-class correlations for C18:1, C18:2, C12:0, sucrose, citric acid, NaCl, caffeine and MSG detection thresholds

Correlation	C18:1	C18:2	C12:0	Sucrose	Citric Acid	NaCl	Caffeine	MSG
Within day	ICC = 0.83 CI = 0.59 – 0.93 *P* < 0.001	ICC = 0.88 CI = 0.71 – 0.95 *P* < 0.001	ICC = 0.80 CI = 0.53 – 0.92 *P* < 0.001	ICC = 0.77 CI = 0.47 – 0.91 *P* < 0.001	ICC = 0.71 CI = 0.40 – 0.80 *P* < 0.001	ICC = 0.71 CI = 0.42 – 0.89 *P* < 0.001	ICC = 0.36 CI = 0.01 – 0.73 *P* < 0.063	ICC = 0.54 CI = 0.11 – 0.79 *P* < 0.009
Across day	ICC = 0.64 CI = 0.58 – 0.86 *P* < 0.001	ICC = 0.60 CI = 0.21 – 0.83 *P* < 0.001	ICC = 0.69 CI = 0.34 – 0.87 *P* < 0.001	ICC = 0.66 CI = 0.29 – 0.86 *P* < 0.001	ICC = 0.87 CI = 0.69 – 0.95 *P* < 0.001	ICC = 0.72 CI = 0.39 – 0.88 *P* < 0.001	ICC = 0.42 CI = 0.36 – 0.73 *P* < 0.03	ICC = 0.78 CI = 0.51 – 0.91 *P* < 0.001
Duplicate across day	ICC = 0.68 CI = 0.31 – 0.87 *P* < 0.001	ICC = 0.88 CI = 0.71 – 0.95 *P* < 0.001	ICC = 0.67 CI = 0.30 – 0.86 *P* < 0.001	ICC = 0.57 CI = 0.15 – 0.81 *P* < 0.006	ICC = 0.95 CI = 0.87 – 0.98 *P* < 0.001	ICC = 0.58 CI = 0.13 – 0.71 *P* < 0.02	ICC = 0.53 CI = 0.11 – 0.80 *P* < 0.009	ICC = 0.85 CI = 0.65 – 0.94 *P* < 0.001
Triplicate across day	ICC = 0.78 CI = 0.49 – 0.91 *P* < 0.001	ICC = 0.94 CI = 0.84 – 0.98 *P* < 0.001	ICC = 0.80 CI = 0.54 – 0.92 *P* < 0.001	ICC = 0.65 CI = 0.27 – 0.85 *P* < 0.001	ICC = 0.96 CI = 0.89 – 0.98 *P* < 0.001	ICC = 0.58 CI = 0.12 – 0.71 *P* < 0.02	ICC = 0.54 CI = 0.12 – 0.80 *P* < 0.008	ICC = 0.83 CI = 0.60 – 0.93 *P* < 0.001
Day within subject	ICC = 0.77 *P* < 0.001	ICC = 0.61 *P* < 0.001	ICC = 0.66 *P* < 0.001	ICC = 0.51 *P* < 0.006	ICC = 0.92 *P* < 0.001	ICC = 0.59 *P* < 0.02	ICC = 0.30 *P* < 0.04	ICC = 0.61 *P* < 0.002

C18:1, oleic acid; C18:2, linoleic acid; C12:0, lauric acid; NaCl, sodium chloride; ICC, intra-class correlation; CI, confidence interval

Figure 4.2: Detection threshold plots of each individual for **A.** oleic acid, **B.** linoleic acid and **C.** lauric acid for the average of the first three sessions versus the average of the second three sessions

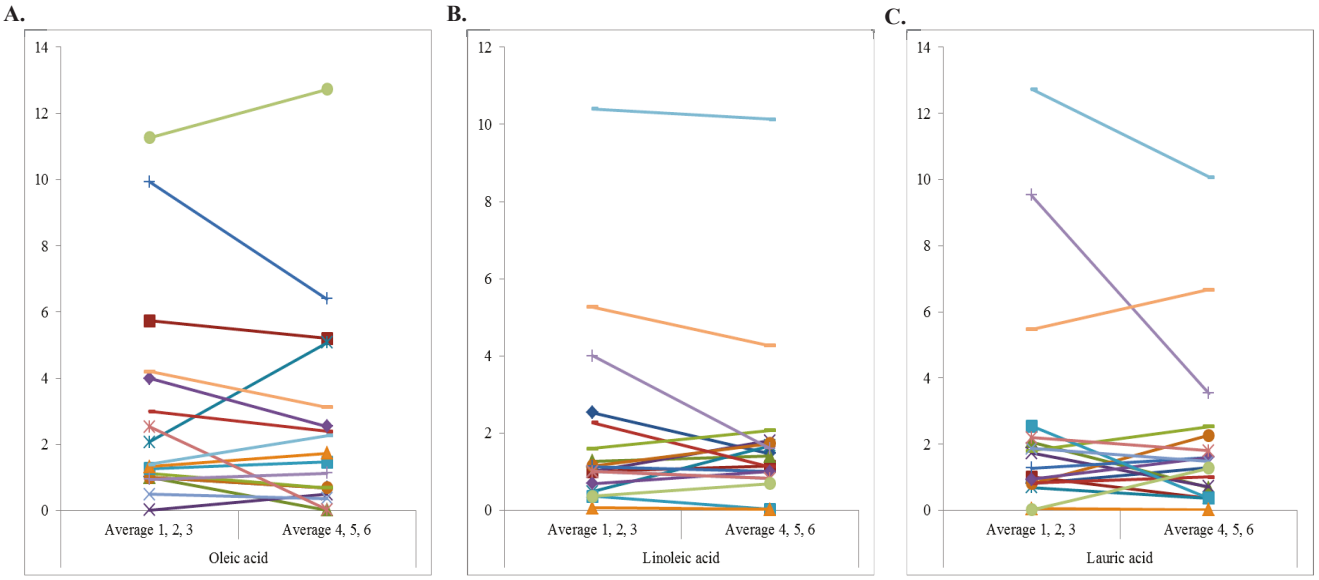
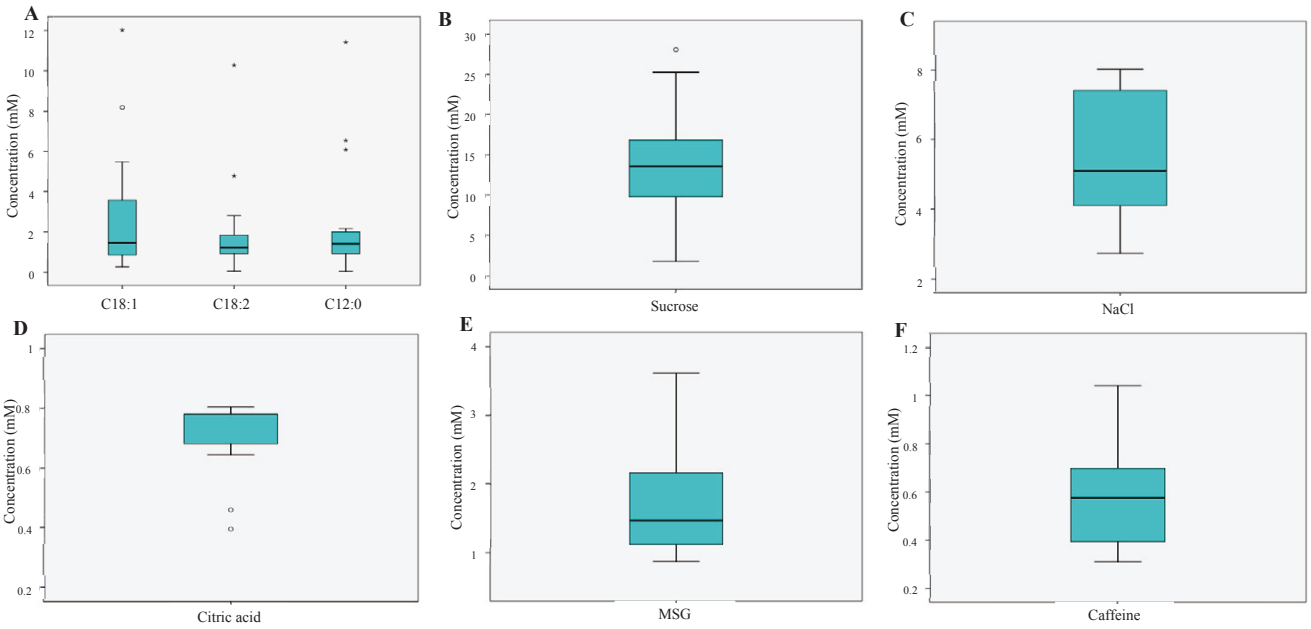


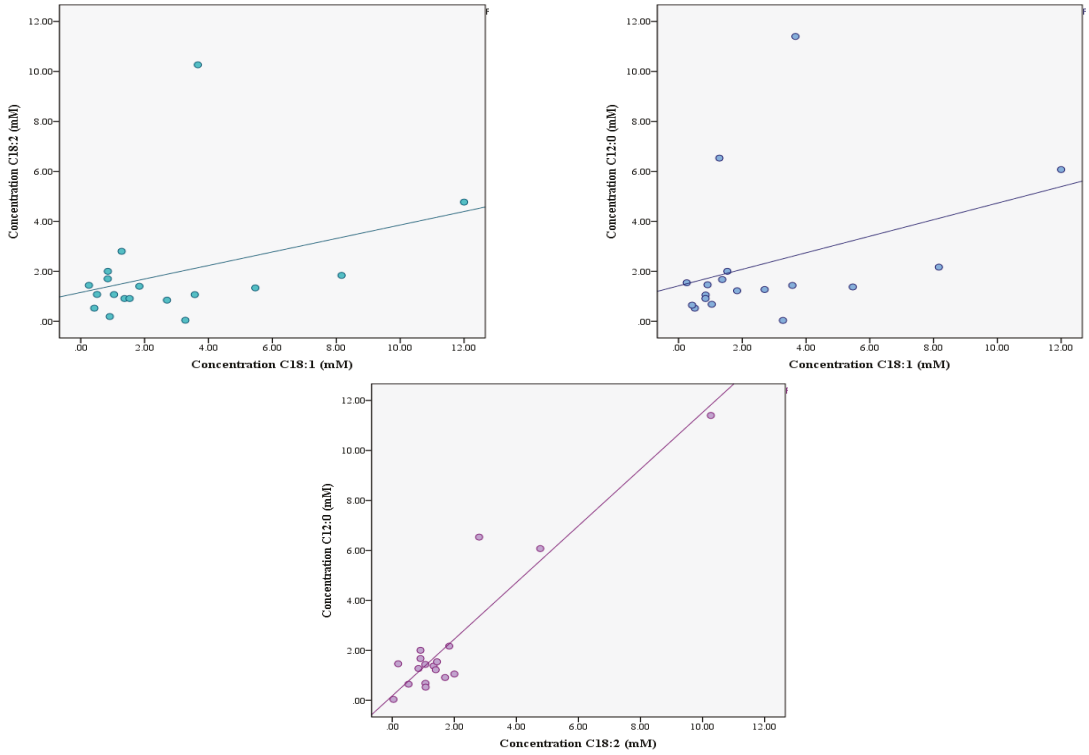
Figure 4.3: Box plots representing distributions of taste detection thresholds. Medians are represented by the line inside the rectangle, the upper and lower quartiles are represented by the 'whiskers' and the dots represent the outliers (○ more or less than 1.5 box-lengths from the edge of the box, * more than 3 box-lengths from the edge of the box). **A**, Detection threshold distribution of C18:1, C18:2, C12:0. **B**, Detection threshold distribution of sucrose. **C**, Detection threshold distribution of NaCl. **D**, Detection threshold distribution of citric acid. **E**, Detection threshold distribution of MSG. **F**, Detection threshold distribution of caffeine.



Associations between C18:1, C18:2 and C12:0 detection thresholds

Moderate associations between C18:1 and C18:2 were found when the average of sessions one to six were compared ($r = 0.65$, $P < 0.004$). Moderate associations were found between C18:1 and C12:0 ($r = 0.50$, $P = 0.037$) and strong correlations were found between C18:2 and C12:0 ($r = 0.82$, $P = 0.001$) **(Figure 4.4)**.

Figure 4.4: Correlations between oleic acid; C18:1, linoleic acid; C18:2 and lauric acid; C12:0. Significant associations were detected through Pearson's correlation analysis



4.5.3 Taste detection thresholds: sweet, sour, salty, bitter and umami

Detection thresholds for all taste qualities were established for each participant (sucrose, 13.61 ± 1.67 mM [range 1.78-28.04 mM], citric acid, 0.71 ± 0.03 mM [range 0.4-0.8 mM], NaCl, 5.48 ± 0.41 mM [range 2.74-8.01 mM], caffeine, 0.58 ± 0.05 mM [range 0.31-1.04 mM], MSG, 1.76 ± 0.19 mM [range 0.88-3.60 mM]) **(Figure 4.3)**. For each subject there was no significant differences found between sucrose, citric acid, NaCl, caffeine and MSG for sessions one, two and three on days one and two ($P = 0.345$, $P = 0.324$, $P = 0.340$, $P = 0.093$, $P = 0.121$, respectively). For sucrose, strong correlations were found for within day testing sessions one and two (ICC = 0.77, CI = 0.47-0.91, $P < 0.001$) and moderate correlations for across day testing sessions one and four (ICC = 0.66, CI = 0.29-0.86, $P < 0.001$). Duplicate testing across days was also moderately correlated, as was across day triplicate testing. For citric acid, strong correlations were found for within day testing sessions one and two (ICC = 0.87, CI = 0.69-0.95, $P < 0.001$) and across day testing sessions one and four. Duplicate testing across days was also strongly correlated, as was across day triplicate testing. The relationship between NaCl detection thresholds on days one and two in session two were strongly correlated (ICC = 0.71, CI = 0.42-0.89, $P < 0.001$). Weak to moderate correlations were found for within day testing sessions one and two for caffeine (ICC = 0.36, CI = 0.10-0.80, $P < 0.063$), but across day testing sessions one and four were moderately correlated. Duplicate testing across days was also strongly correlated, as was across day triplicate testing. Lastly, for MSG moderate correlations were found within day testing sessions one and two (ICC = 0.54, CI = 0.11-0.79, $P < 0.009$), but strong correlations were found between across day testing sessions one and four. Duplicate testing across days was also

strongly correlated, as was across day triplicate testing. Again, moderate to strong correlations for sucrose, citric acid, NaCl, caffeine and MSG were found when comparing thresholds of one individual across the two days **(Table 4.1)**.

4.5.4 Food consumption

From the daily food records, it was found that there were no significant correlations between C18:1, C18:2 and C12:0 detection thresholds and total energy intake (P = 0.33, P = 0.81, P = 0.62), total fat (P = 0.53, P = 0.65, P = 0.75), saturated fat (P = 0.90, P = 0.77, P = 0.77), polyunsaturated fat (P = 0.21, P = 0.89, P = 0.88) and monounsaturated fat intake (P = 0.61, P = 0.13, P = 0.78) and total carbohydrate (P = 0.31, P = 0.79, P = 0.58) and protein intake (P = 0.37, P = 0.67, P = 0.66), respectively.

4.5.5 Anthropometry

The mean BMI was 22.9 ± 0.75 kg/m^2. There was no significant relationship between fatty acid detection thresholds and BMI for C18:1 (r = -0.14, P = 0.59), C18:2 (r = -0.06, P = 0.83) or C12:0 (r = -0.19, P = 0.46).

4.6 Discussion

This study investigated the reproducibility of oral fatty acid detection thresholds. Oral fatty acid detection thresholds were reliable and reproducible both within and across days for C18:1, C18:2 and C12:0. The test-retest coefficients for fatty acids were equivalent to sucrose (sweet), citric acid (sour), caffeine (bitter), MSG (umami) and NaCl (salty) (Ahne, et al., 2000; Pingel et al., 2010). The current study has

shown that an individuals' oral fatty acid detection thresholds were as consistent and reproducible over numerous sessions as oral detection thresholds for the sweet, sour, salty, bitter and umami stimuli (Ahne, et al., 2000; Bachmanov & Beauchamp, 2007; Cicerale, et al., 2009; Galindo-Cuspinera et al., 2009; Krarup, 1958; Linschoten, Harvey, Eller, & Jafek, 2001; Mueller, et al., 2011).

An important finding from this study was that the reproducibility of both the saturated and unsaturated oral fatty acid thresholds measured was moderate to strong (ICC range = 0.6-0.94), indicating that they remained stable within and across testing sessions and days of testing. This study showed that duplicate threshold measurements on the same day may be used to achieve an accurate portrayal of an individual's fatty acid sensitivity and that triplicate measures within or across days did not provide any extra accuracy to the measures. Furthermore, although correlations for duplicate measures across days for all three fatty acids were moderate in strength, duplicate measures on the same day appear to provide the most convenient option for testing oral fatty acid detection thresholds. However, not all studies agree with this. Running et al. (2013) believe that there is a learning effect related to fatty acid threshold testing with their study finding that subjects took up to seven sessions before they could pick the fatty acid sample consistently (Running, et al., 2013). However, this contradicts what was found in the present study, with a proposition that duplicate sessions are needed to gain an accurate portrayal of an individual's oral fatty acid sensitivity. Reasons for these differences between studies may be due to differences in the vehicle used to present the fatty acid samples in; water compared to non-fat milk samples and differences in methodology; ascending

3-alternate forced choice (3-AFC) methodology and staircase method compared to triangle tests with ascending forced choice methodology used in the present study (Newman & Keast, 2013; Tucker & Mattes, 2013). Other potential explanations for differences in results include the fact that subjects completing the staircase method started testing at a mid-range concentration of 3.2 mM which may have left residual stimuli, and therefore made it difficult for subjects to detect the fatty acids at lower concentrations (O'Mahony, 1979). Additionally, after the first threshold measure had been established using the 3-AFC methodology, subjects began the next testing session six concentration steps below their previously obtained threshold (Tucker & Mattes, 2013), whereas the triangle tests in the present study began at the same concentration (0.02 mM) for each testing session (Newman & Keast, 2013). These differences in starting position may explain the variability of thresholds obtained using the staircase and 3-AFC methods compared to the reliability reported using the triangle tests.

As expected in sensory modalities, oral fatty acid thresholds varied considerably among individuals tested and this explains the fact that there were outliers for the three fatty acids. Oral detection thresholds ranged from 0.04 mM to 12 mM across all fatty acids. Intra-individual variability in oral fatty acid sensitivity has been observed in prior investigations (Chale-Rush, et al., 2007a; Mattes, 2009c; Stewart, et al., 2010; Stewart, Newman, et al., 2011; Stewart, Seimon, et al., 2011) and results from the present study have indicated measurement variation is a minor factor as thresholds remained stable during testing sessions.

Strong correlations were found between all three fatty acids, meaning that if an individual was less orally sensitive to C18:1, they were also more likely to be less orally sensitive to C18:2 and C12:0. Previously, studies have also found strong correlations between C18 unsaturated fatty acids, suggesting a common oral transduction mechanism may be at play (Stewart, et al., 2010). Indeed, reports have located GPCR40 and GPCR120 on taste cells (Ichimura, et al., 2012), which bind fatty acids upon chain length (Tanaka et al., 2008). Fatty acids that are between 6-12 carbon atoms long are classified as medium-chain fatty acids, whereas those that are greater than 18 carbon atoms long are long-chain fatty acids (Beermann et al., 2003). GPCR40 and GPCR120 are activated by medium- and long-chain fatty acids (Tanaka, et al., 2008), therefore, as all three fatty acids were strongly correlated with one another, it can be suggested that C18:1, C18:2 and C12:0 may access a similar transduction mechanism to each other (Keast & Breslin, 2002).

Intra-individual variability in the primary tastes and oral fatty acid sensitivity have been observed in prior investigations (Bertino, Beauchamp, & Engelman, 1986; Chale-Rush, et al., 2007a; Delwiche, Buletic, & Breslin, 2001; Eny, Wolever, Fontaine-Bisson, & El-Sohemy, 2008; Mattes, 2009c; Stewart, et al., 2010), however the factors responsible for variability remain undisclosed. The current study has indicated measurement variation is a minor factor as thresholds remained stable during testing sessions. It is however known that sensitivity to tastants and possibly fatty acids, may vary due to environmental factors, such as the background diet (DiNicolantonio, Teow, & Morgan, 1984), or genetic factors, such as differences in taster status (*e.g.,* supertaster) (Hayes & Keast, 2011; Hayes, Sullivan, & Duffy,

2010) or taste cell receptor density (Tepper & Nurse, 1997), which may influence taste cell functionality (DiNicolantonio, et al., 1984).

It is also important to note that although taste thresholds for the three fatty acids and five taste primaries were equivalent, two different vehicle compositions were used; fatty acids were presented in non-fat milk and the primary tastes were presented in distilled water. Reasons for using non-fat milk include the fact that it is a realistic food matrix in which fats are normally found in the food supply and to ensure homogeneity of all samples as this can be hard to achieve in oil in water emulsions. However, the matrix of milk is more complex than that of water, therefore, the use of milk may potentially cause an increase in background sensory noise and influence a subject's ability to detect the fatty acids in this background, compared to the primary taste qualities in water (Running, et al., 2013). Nonetheless, as fatty acid detection thresholds were found to be as reliable as those of the primary tastes, it could be suggested that the vehicle used may not be a major factor contributing to variability in oral fatty acid detection thresholds; in fact this particular vehicle may assist by decreasing background noise.

The extent that diet plays in inter-individual variability in fatty acid thresholds remains unknown. We did not find a relationship between reported dietary fat intake and oral fatty acid sensitivity, in agreement with other studies (Tucker, Laguna, Quinn, & Mattes, 2013; Tucker & Mattes, 2013). This may be as a result of subjects only recording their dietary intake for the morning of testing as opposed to a 24-hour food intake or measures of habitual diet. This study did not find a relationship

between oral fatty acid sensitivity and BMI as has been previously reported by some researchers (Stewart, et al., 2010; Stewart, Newman, et al., 2011). This may be due to the small sample size which would have decreased the variability in BMIs between subjects and therefore decreased the chance of finding a statistical association. Studies which have previously found associations have used much larger sample sizes (greater than 50 subjects) (Stewart, et al., 2010; Stewart, Newman, et al., 2011) and as the main outcome of this study was to determine reliability, the 18 subjects did not provide adequate statistical power to determine associations between oral sensitivity and BMI. In addition, the majority of subjects were of a healthy BMI, with three out of 18 subjects classified as overweight or obese. Therefore the variability amongst participants with regards to BMI classification was decreased and thus decreased the chance of finding an association. Moreover, previous studies which have found an association between oral fatty acid sensitivity and BMI have used an abbreviated screening procedure and classification system to categorise subjects as hypersensitive or hyposensitive, whereas those who did not find an association used a complete threshold testing procedure (Kamphuis, Saris, et al., 2003; Mattes, 2009d, 2011b; Stewart & Keast, 2012), as was used in the present study. The variance amongst subjects with regards to oral fatty acid detection thresholds cannot be seen when using the classification method, therefore relationships found between oral fatty acid sensitivity and BMI may be overstated. Nevertheless, the associations between fatty acid taste thresholds and overweight/ obesity remain intriguing and future studies specifically designed around this question are needed.

4.7 Conclusions

In summary, this study has provided confirmatory evidence in support of the good test-retest reliability of sweet, sour, salty, bitter and umami thresholds, as well as novel evidence showing the high reproducibility of oral fatty acid thresholds using saturated, monounsaturated and polyunsaturated fatty acids. The fact that there were no correlations between fatty acid thresholds and primary taste thresholds adds to the growing body of evidence that suggest fatty acids may have an independent taste transduction mechanism. These findings suggest that the most accurate way to test an individual's oral sensitivity to C18:1, C18:2 and C12:0 are to perform the tests in duplicate on the same day. This method provides a convenient and precise way to measure an individual's oral sensitivity to fatty acids.

4.8 Future directions

Due to the paucity of evidence in humans with regards to oral fatty acid sensitivity and putative receptor expression, future research should focus on this area. Studies that involve assessing an individual's oral fatty acid sensitivity over a number of sessions are required, which also yield a tongue papillae biopsy to investigate associations with putative receptors and pre-determined oral fatty acid sensitivity.

CHAPTER FIVE

Study 2

The influence of a high-fat meal immediately prior to oral fatty acid detection threshold testing

5.1 Introduction

Taste is used as an oral nutrient-toxin detection system which is designed to detect the nutritional or toxic quality of foods and ensure essential nutrients vital for survival and functioning are consumed and reject foods that may be harmful (Gilbertson, et al., 2000). The detection of compounds within the oral cavity can have an appetitive effect or an aversive effect, for example, sweet taste indicates the presence of carbohydrates and umami generally indicates proteins, which both evoke an appetitive response, however sour and bitter may indicate the presence of potential toxins or poisons, and therefore produce an aversive response (Gilbertson, et al., 2000). As the oral cavity detects the major macronutrients of the human diet, *e.g.,* carbohydrates and proteins, research is now suggestive that there may also be detection of fats within the oral cavity which is activated by the break down product of fat, fatty acids.

Historically, it was thought that fats could be perceived across multiple modalities including olfaction, texture, chemesthesis, and vision (Tucker & Mattes, 2012), however research is now suggesting that when non-taste cues are minimised, fatty acids can be identified by humans at detection threshold levels within the oral cavity (Chale-Rush, et al., 2007a; Mattes, 2009c; Stewart, et al., 2010; Stewart, Newman, et

al., 2011). Further to this, specific putative receptors cluster of differentiation 36 (CD36), G-protein coupled receptor (GPCR) 120, 40 and 43 and delayed rectifying potassium (DRK) channels for fatty acids have been identified in the oral cavity on taste tissue in animals (Ichimura, et al., 2012; Sclafani, Ackroff, & Abumrad, 2007; Simons, et al., 2010). However, at this stage, only GPCR 120 and CD36 have been isolated in human taste tissue (Galindo, et al., 2012). Nonetheless, these findings add to the accumulating body of evidence for fatty acid detection within the mouth.

Mounting evidence is suggestive of a strong link between dietary fat consumption and sensitivity to fatty acids. Research has focussed on the potential link between excess dietary fat consumption and sensitivity to fatty acids throughout the alimentary canal (Little & Feinle-Bisset, 2011; Stewart, Seimon, et al., 2011). Recent work has found that when subjects are grouped based on their sensitivity to oleic acid (C18:1), hypersensitive subjects consumed less total energy and fat and were more likely to perform behaviours that reduced their fat consumption *e.g.,* trimming the fat off meat and avoiding saturated fats, than hyposensitive subjects (Stewart, et al., 2010; Stewart, Newman, et al., 2011). However, the mechanisms responsible for these differences between hypersensitive and hyposensitive individuals remain elusive. This work also suggested that oral fatty acid sensitivity was linked to body mass index (BMI) with hypersensitive individuals having lower BMIs than hyposensitive individuals. Nevertheless, this link remains contentious with many other studies finding no link between BMI and oral fatty acid sensitivity (Kamphuis, Saris, et al., 2003; Mattes, 2009d, 2011b; Stewart & Keast, 2012). Suggestions as to why this may be the case have been proposed with the thought using an abbreviated

screening procedure for testing oral fatty acid sensitivity *i.e.,* splitting the subjects into hypersensitive and hyposensitive based on oral sensitivity to a single C18:1 concentration has played some form of role, as all studies that have used a full screening procedure *e.g.,* 3-alternate forced choice (3-AFC), triangle tests or the staircase methodology have found no links between oral sensitivity and BMI (Running, et al., 2013). In addition, studies that have divided subjects into lean and obese groups have also found mixed results with one study finding a strong correlation between BMI and oral fatty acid sensitivity (Stewart, Seimon, et al., 2011) and the other finding no correlations (Stewart & Keast, 2012), which provides further evidence to suggest that this is a contentious area of research. It is still speculative at this stage as to what the driving factor of excess fat consumption is; does sensitivity drive fat consumption, or is fat consumption determining sensitivity. The modulation of oral fatty acid thresholds was investigated in relation to fat consumption (Stewart & Keast, 2012). Subjects followed a low-fat diet for four weeks and then followed a high-fat diet for four weeks. After the low-fat diet, lean and overweight/ obese subjects' sensitivity to fatty acids increased and after consumption of the high-fat diet, the lean subjects' fatty acid sensitivity decreased (Stewart & Keast, 2012). These findings highlight the potential link between fat consumption and oral fatty acid sensitivity. However, interestingly, after consumption of the high-fat diet, it was reported that oral fatty acid thresholds did not change in the obese population. Suggestions for this seemingly differential response included, the proposal that the obese population were consuming a high-fat diet prior to commencement of the study, and therefore adaptation to this diet had already occurred, in turn causing no changes in sensitivity. Nevertheless, no

significant differences in sensitivity were seen at baseline between the lean and obese groups, thus if a reduction in fat consumption could be maintained over the long-term, adaptive changes to sensitivity may occur whereby subjects will become more sensitive to the physiological and psychological effects of fat, and in turn require smaller amounts of fat to elicit the positive post-ingestive effects associated with fats.

Whilst there is speculation over the reliability of the fatty acid detection measurement, recent work has established that the method we employ is highly reproducible over a number of testing sessions (Newman & Keast, 2013). The study found that the most reliable and highly correlated measurement to ascertain the most accurate portrayal of a subject's sensitivity to C18:1, linoleic acid (C18:2), lauric acid (C12:0), was to perform the threshold test in duplicate. In contrast, recent work has also reported that taste thresholds are not stable from session to session with an observed learning effect occurring (Tucker & Mattes, 2013). The authors report that up to seven sessions would be needed to gain an accurate portrayal of an individual's sensitivity, whilst Newman and Keast (2013) recommend sessions to be completed in duplicate. Reasons for these discrepancies may be due to differences in testing method used (ascending forced choice triangle tests compared to staircase method and 3-AFC methodology) and vehicle used to present the fatty acid samples in (non-fat milk compared to water).

Evidence is now emerging that oral fatty acid sensitivity may be influenced by long-term fat consumption; however it is unknown whether recent intake could contribute

to intra-individual variation with regards to oral fatty acid detection thresholds. Due to the regularity of taste cell turnover; approximately every 10-14 days (Farbman, 1980), it could be speculated that a diet high or low in fat would need to be consumed for at least 10-14 days before changes in peripheral physiology could be seen. However, to the best of the author's knowledge, it is unknown if a high-fat meal immediately prior to threshold testing has any effect on oral fatty acid detection threshold performance. Some research has been conducted in animals whereby CD36 mRNA levels decreased in fed mice in comparison to the fasted mice and in mice who had consumed a high-fat diet compared to those on a non-fat diet (Martin et al., 2011) suggesting the prior meal may have an influence on taste sensitivity. This study also suggests that this decrease in CD36 was mediated by lipids as decreases were only seen when the chow contained long-chain fatty acids (Martin, et al., 2011). The authors also postulate that these decreases may be due to CD36 degradation, whereby a negative feedback response to persistent exposure to dietary fats may cause a physiological desensitisation of lingual CD36 (Martin, et al., 2011). Therefore, it is unknown whether this is the case in humans, but perhaps these acute decreases in CD36 after fat exposure may influence the ability to detect fatty acids within the oral cavity. However, there are conflicting results in humans regarding the effect of a meal on taste thresholds with past research reporting that consumption of a high-carbohydrate meal influences taste perception of sweet taste in men, but not women (Suchecka et al., 2011). The same study also reported that salty taste was not influenced by the high-carbohydrate meal (Suchecka, et al., 2011), suggesting that it may be a macronutrient specific effect. Similarly, another study reported that there was a trend for a reduced taste of sucrose after a meal (Moore, Linker, & Purcell,

1965). Other studies have investigated the effect of a test meal on detection and recognition thresholds for the primary tastes and found no differences in taste thresholds for sucrose, fructose, sodium chloride (NaCl), 6-*n*-propylthiouracil (PROP) and quinine pre- and post-meal (Pasquet et al., 2006). This may be due to the fact that a once off exposure to the potential tastants or macronutrients within a single meal is not enough exposure to elicit a change in detection thresholds and perhaps repeated or long-term exposure is needed to see such changes, as reported in dietary studies (Mattes, 1997; Stewart & Keast, 2012). In contrast, Zverev (2004) reported that there were differences in taste thresholds for sucrose, salt and quinine pre- and post-meal, however the pre-meal threshold was taken after 14 hours of fasting, whereas generally subjects would be required to refrain from eating or drinking 1-2 hours prior to testing and perhaps this may account for differences between studies (Zverev, 2004). The composition of the meals provided to the subjects in both studies may also impact on the differing results found, with many studies not providing this information for comparison. Thus, the contradictory background research in this area and the fact that this has not been investigated in regards to oral fatty acid sensitivity suggest that it is an area worth exploring.

5.2 Aims and hypotheses

5.2.1 Aims

To measure the effect that a high-fat (60% fat: 20% carbohydrate: 20% protein), a low-fat (20% fat: 40% carbohydrate: 40% protein) or a macronutrient balanced (33% fat: 33% carbohydrate: 33% protein) meal immediately prior to detection threshold testing has on oral fatty acid thresholds.

5.2.2 Hypotheses

The consumption of a high-fat, low-fat or macronutrient balanced meal prior to threshold testing will have no effect on oral fatty acid detection thresholds as physiological adaptation is unlikely to occur during a single meal.

5.3 Subjects, materials and methods

5.3.1 Subjects

Power analysis was conducted prior to the study to determine an appropriate sample size to achieve adequate statistical power. Data were used from a prior study which evaluated detection thresholds for fatty acids (Stewart, et al., 2010). Using an α level of 0.05 and a 10% β (90% power), it was predicted that 31 subjects would be required for this study to detect a C18:1 detection threshold difference of 0.65 mM, using the following equation:

$$N = \frac{(Z_\alpha + Z_\beta)^2 * \sigma^2}{D^2}$$

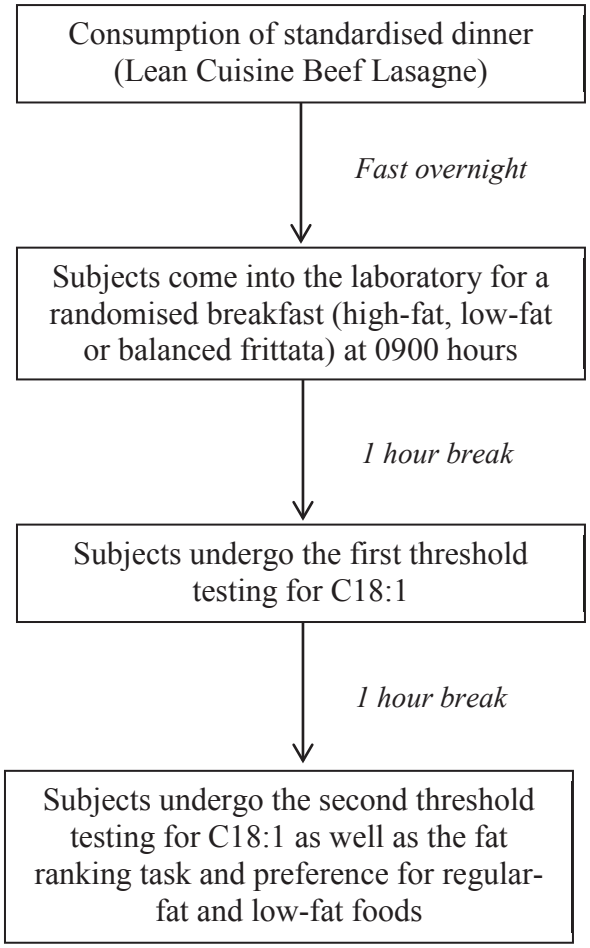
Where N represents the sample size, α represents the probability of a type I error (Z = 1.96, 95% confidence), β represents the probability of a type II error (Z = 1.64, 90% confidence), σ represents the standard deviation and D is the minimal difference required for detection of significant differences. Subjects were recruited from Deakin University, Burwood, Victoria, Australia and the surrounding suburbs. Subjects were required to be non-smokers and aged between 18-75 years old. This

study was approved by the Deakin University Human Ethics Advisory Group (HEAG-H20_2012) and all subjects provided informed, written consent prior to participating in the study.

5.3.2 Study outline

This study was a randomised crossover study where subjects were exposed to all treatments. Subjects were required to attend three laboratory sessions at the Deakin University sensory laboratory. On the evening prior to each testing session, subjects were asked to consume a standardised meal (Lean Cuisine Beef Lasagne, Simplot, Mentone, Victoria, Australia). In each session, subjects attended the laboratory at the same time (0900hr) where they consumed one of the three breakfasts; a high-fat, low-fat or equal fat, protein and carbohydrate breakfast **(Appendix H)**. After a one hour break, they were tested for their oral sensitivity to C18:1 in duplicate **(Figure 5.1)**. Anthropometric measurements were also recorded to calculate BMI and subjects were required to complete a food frequency questionnaire and a 24-hour food record.

Figure 5.1: Overview of the study design



5.3.3 C18:1 samples

Food grade C18:1 was added to long-life skim milk samples at varying concentrations, as per previous research (Newman & Keast, 2013; Stewart, et al., 2010; Stewart, Newman, et al., 2011) (outlined in **Chapter 3**). All samples were mixed with 5% gum acacia and liquid paraffin to minimise textural differences. Ethylenediaminetetraacetic acid (EDTA) (0.01% w/v) was also added to all samples to prevent oxidation. A stable emulsion was created by combining the gum, EDTA

and milk together first and then this was added to the paraffin and fatty acid mixture **(Chapter 3)**. Control samples were made in the same way as the fatty acid samples, minus the C18:1. Samples were homogenised at 12 000 rpm for 30 seconds/ 100 ml. All samples were made freshly on the day of testing.

5.3.4 Detection thresholds for C18:1

Oral fatty acid sensitivity to C18:1 was determined using triangle tests with ascending forced choice methodology **(Chapter 3)**. Subjects were presented with three samples on a tray; two were control samples and the other containing a set concentration of C18:1. Fatty acid samples were presented in ascending order from the lowest concentration (0.02 mM) to the highest (20 mM) (Newman & Keast, 2013; Stewart, et al., 2010). Detection thresholds for C18:1 were determined when a subject was able to pick the odd sample out three consecutive times at the same concentration. Subjects were instructed to taste the samples and then spit them out. They were also required to wear a nose clip at all times and perform the test under red lighting to minimise potential confounders from non-sensory inputs. Subjects were asked to refrain from eating or drinking anything one hour prior to testing (water was allowed). Detection thresholds were measured in duplicate whereby subjects were tested one hour after breakfast and then one hour after completion of the first threshold test.

5.3.5 Test meals

Subjects were provided with and asked to consume a standardised dinner (Lean Cuisine Beef Lasagne) on the evening prior to testing and were asked not to consume

any other foods (water was allowed) until attending the sensory laboratory the next day **(Chapter 3)**. During all three sessions, subjects were given one of three different frittatas on three separate days, which varied in macronutrient content (high-fat, low-fat, equal amount of fat, protein and carbohydrate) **(Appendix H)** and were then asked to come back twice on the same day to measure oral fatty acid sensitivity. The breakfasts were matched for energy and the kilojoule content for each frittata was 1500kJ. To compensate for variation in the volume of each breakfast, breakfasts were equated with a measured amount of drinking water. Subjects were asked to refrain from eating or drinking anything (except for water) between breakfast and the first oral fatty acid sensitivity test. C18:1 composition of the frittatas was calculated using FoodWorks 2009 (Xyris software, Highgate Hill, Queensland, Australia), RMIT Australian Fatty Acids database.

5.3.6 Fat ranking task

At the end of each laboratory session, subjects were asked to complete a fat ranking task which assessed the subject's ability to identify fats within a common food (custard). As per **Chapter 3**, subjects were presented with custard samples containing varying amounts of vegetable oil (0, 2, 6 and 10%). Custard was made from 20 g custard powder, 12 g sugar and 500 ml skim milk. Custard was then separated into four 100 g batches and 2 ml, 6 ml and 10 ml of vegetable oil was added to the batches and the 0% batch contained no vegetable oil. All samples were matched for texture so they contained a total of 10% 'oil'; therefore paraffin oil was added to the 0%, 2% and 6% batches. Subjects were given the samples in a randomised order and asked to taste each sample and rank the samples in order of

perceived fattiness. Subjects received a score out of five for this task. The scores for this were calculated as per previous research **(Table 3.2)** (Stewart, Newman, et al., 2011).

5.3.7 Hedonic ratings

Subjects completed preference tests using a range of regular-fat and low-fat foods including cream cheese, vanilla yoghurt and chocolate mousse, as per **Chapter 3**. Subjects were asked to taste each sample and rate how much they liked it using a 9-point hedonic scale ranging from 'dislike extremely' to 'like extremely'.

5.3.8 Habitual food consumption

Subjects were required to fill out a food record in which they recorded everything they consumed in 24 hours **(Appendix F)**. Subjects were asked to, where possible, weigh the foods they consumed or use standard metric measuring cups, or common serving sizes, for example, 1 cup. Diet diaries were analysed using FoodWorks 2009 (Xyris software, Highgate Hill, Queensland, Australia). From these data the mean energy intake (kJ), macronutrient distribution (% energy from fat, protein and carbohydrate and grams of fat, protein and carbohydrate) and the type of fat (grams and % of monounsaturated, polyunsaturated or saturated), salt (mg) and alcohol (g) was calculated.

5.3.9 Anthropometry

Height (m) and weight (kg) measurements were taken for all subjects using a stadiometer (Seca, MedShop Australia, Fairfield, Victoria, Australia) and scales

(Tanita Body Scan Composition Monitor Scales, Cloverdale, Western Australia, Australia), respectively. Light loose clothing was worn and shoes were removed **(Chapter 3)**.

5.4 Statistical analysis

Differences in oral fatty acid sensitivity after each breakfast were compared using one-way repeated measures analysis of variance (ANOVA). Each session was numbered according to **Table 5.1**. Within day differences in oral fatty acid sensitivity were determined using paired samples t-tests. Reliability analysis was conducted using intra-class correlations (ICC) to detect associations between C18:1 detection thresholds within and between testing days. One-way repeated measures ANOVA was also used to detect differences between preferences for regular- and low-fat foods after each breakfast and Friedman tests were used to detect differences between fat ranking scores after each breakfast. Independent samples t-tests were used to detect differences in oral fatty acid sensitivity, gender and healthy weight category as well as differences in energy and macronutrient intake between lean and obese subjects. Pearson's correlations were used to detect associations between C18:1 detection thresholds and total energy, fat, carbohydrate and protein intake, as well as percentage energy from fat, carbohydrates and proteins. All data are presented as means ± SEM.

Table 5.1: Number of testing days and sessions; subjects came in for six sessions over three days

Day	Session number
1	1 and 2
2	3 and 4
3	5 and 6

5.5 Results

5.5.1 Subjects

Subjects (*n* = 32, 15 males: age 49.3 ± 4.8 years [range 22-73 years], BMI 24.7 ± 0.8 kg/m^2 [range 20.8-30.5 kg/m^2], 17 females: age 31.5 ± 2.8 years [range 22-60 years], BMI 21.86 ± 0.9 kg/m^2 [range 18.3-33.8 kg/m^2]) were recruited from Deakin University and surrounding areas. Out of the total 32 subjects, 7 were classified as overweight/ obese (*n* = 7, 5 male, 2 female, BMI 29.1 ± 0.8 kg/m^2 [range 25.5-33.7 kg/m^2]).

5.5.2 C18:1 concentrations of test meals

The high-fat frittata contained 10.1 g (10 104 mg) of C18:1 per serve, the balanced frittata contained 6.17 g (6174 mg) per serve and the low-fat frittata contained 2.30 g (2304 mg) per serve.

5.5.3 Detection thresholds for C18:1

Post-breakfast

Oral detection thresholds were established by all subjects in each session for C18:1. There were no significant differences found in oral detection thresholds after the high-fat, low-fat or macronutrient balanced breakfasts in sessions 1, 3 and 5 (the first testing session of each day) (main effect of time: Wilks' Lambda = 0.920, $F(2, 30) = 1.30$, $P = 0.288$). There were also no significant differences found after the three breakfasts for sessions 2, 4 and 6 (the second session of each day) (main effect of time: Wilks' Lambda = 0.981, $F(2, 29) = 0.285$, $P = 0.754$) **(Table 5.2)**. Although the second threshold measurement of each day was lower, there were no significant differences found between sessions 1 and 2 ($P = 0.198$), sessions 3 and 4 ($P = 0.199$) and sessions 5 and 6 ($P = 0.125$).

Table 5.2: Mean C18:1 detection thresholds after the high-fat, low-fat and macronutrient balanced frittata

Session	HF frittata		LF frittata		B frittata		*P* value
	Mean (mM)	95% CI	Mean (mM)	95% CI	Mean (mM)	95% CI	
1	1.23	0.67-2.27	0.98	0.37-1.66	1.75	0.81-2.37	0.288
2	0.79	0.48-2.04	0.65	0.31-1.37	0.99	0.54-2.04	0.754

1, threshold session one; 2, threshold session two; CI, confidence interval; HF, high-fat; LF, low-fat; B, balanced

P values represent main effect of time using repeated measures ANOVA

Reliability

Moderate to strong correlations were found for within day testing after each breakfast (HF: ICC = 0.63, CI = 0.23-0.82, $P = 0.004$; LF: ICC = 0.48, CI = -0.07-0.75, $P = 0.037$; B: ICC = 0.91, CI = 0.81-0.96, $P < 0.001$) meaning the measure was reliable. There were also strong correlations across day testing for sessions 1, 3 and 5 (ICC = 0.83, CI = 0.70-0.91, $P < 0.001$), conversely a weak correlation was found between sessions 2, 4 and 6 (ICC = 0.27, CI = -0.33-0.62, $P = 0.15$) meaning that these sessions were not highly reliable.

Lean vs. obese

There was no significant difference in oral fatty acid thresholds found between lean and overweight/ obese subjects, although obese subjects had higher thresholds (lean: 0.88 ± 0.24 mM, obese: 1.63 ± 0.08 mM, $P = 0.326$).

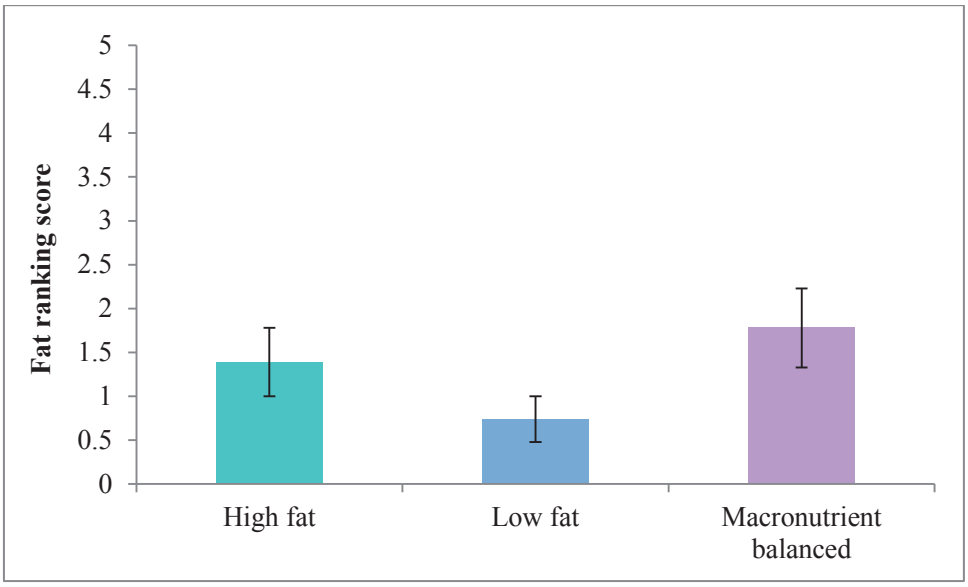
Gender

There were also no significant differences in oral fatty acid sensitivity between males and females (males: 0.81 ± 0.15 mM, females: 1.22 ± 0.10 mM, $P = 0.343$).

5.5.4 Fat ranking

There were no significant differences between fat ranking scores after the high-fat, low-fat or macronutrient balanced breakfasts χ^2 ((2, $n = 22$) = 2.13, $P = 0.345$) (HF, 1.95 ± 0.39; LF, 1.84 ± 0.26; B, 2.20 ± 0.45) **(Figure 5.2)**.

Figure 5.2: Differences in scores for the fat ranking task after the high-fat, low-fat and macronutrient balanced breakfasts



5.5.5 Preferences

There were no significant differences between liking of the regular-fat and low-fat cream cheese (RF: Wilks' Lambda = 0.846, $F(2, 20) = 1.83$, $P = 0.187$; LF: Wilks' Lambda = 0.955, $F(2, 20) = 0.469$, $P = 0.632$) yoghurt (RF: Wilks' Lambda = 0.948, $F(2, 20) = 0.548$, $P = 0.586$; LF: Wilks' Lambda = 0.905, $F(2, 20) = 1.05$, $P = 0.370$) or mousse (RF: Wilks' Lambda = 0.986, $F(2, 20) = 0.143$, $P = 0.868$; LF: Wilks' Lambda = 0.942, $F(2, 20) = 0.650$, $P = 0.532$) after the three breakfast types **(Table 5.3)**.

Table 5.3: Acceptance changes (Mean ± SEM) using a 9-point hedonic scale in regular- and low-fat foods following consumption of the high-fat, low-fat and macronutrient balanced frittatas

Food	High-fat frittata	Low-fat frittata	Balanced frittata	*P* value
RF chocolate mousse	7.22 ± 0.24	7.18 ± 0.20	7.31 ± 0.23	0.868
LF chocolate mousse	6.61 ± 0.36	6.61 ± 0.28	6.30 ± 0.25	0.532
RF yoghurt	6.95 ± 0.29	6.63 ± 0.32	6.73 ± 0.29	0.586
LF yoghurt	6.77 ± 0.29	6.95 ± 0.26	6.50 ± 0.31	0.370
RF cream cheese	6.73 ± 0.20	6.68 ± 0.26	6.50 ± 0.24	0.632
LF cream cheese	6.20 ± 0.29	6.64 ± 0.24	6.52 ± 0.24	0.187

RF, regular-fat; LF, low-fat

P values represent main effect of time using repeated measures ANOVA

5.5.6 Habitual food consumption

From the daily food records there were no significant correlations between oral fatty acid sensitivity to C18:1 and total energy intake (r = 0.080, P = 0.667), total fat intake (r = 0.019, P = 0.920), saturated fat intake (r = 0.038, P = 0.837), carbohydrate intake (r = 0.086, P = 0.647) or protein intake (r = 0.077, P = 0.679). There were also no significant differences in total energy intake, macronutrient intake or percentage macronutrient intake between lean and obese subjects (P > 0.05).

5.5.7 Anthropometry

The mean BMI was 23.2 ± 0.67 kg/m^2. There was no significant association between fatty acid detection thresholds and BMI for C18:1 after any of the breakfasts (HF: r = 0.303, P = 0.064; LF: r = 0.094, P = 0.611; B: r = 0.288, P = 0.110).

5.6 Discussion

This study demonstrated that there was no significant change to C18:1 detection thresholds after consumption of a high-fat, low-fat or macronutrient balanced breakfast. This study also added to the knowledge base of the reliability of the oral fatty acid threshold measurement with a high test-retest reliability between sessions and across days found, although a decrease in thresholds from session one to session two was seen each day. No differences between sessions were found in the ability of subjects to detect the fat content of custard, or preference for regular- and low-fat foods.

This study investigated the effect of a high-fat meal immediately prior to C18:1 detection threshold testing. The fact that there were no significant differences found between testing days and across testing sessions suggests that short-term fat intake and macronutrient composition does not influence oral fatty acid thresholds. However, the present study only used one type of food, therefore it cannot be conclusively stated that there is no effect. How the test food is prepared and the fatty acid composition may have an effect, thus future research will be needed using different types of foods prepared in various ways to gain full understanding of this topic. Whilst unconfirmed at this stage, it may be long-term fat consumption that

modulates oral fatty acid sensitivity. Previous research has shown that a reduction in dietary fat consumption over as little as a four week period can have significant positive effects on an individual's oral sensitivity to fatty acids (Stewart & Keast, 2012). This suggests that prolonged consumption of a low-fat diet may promote habituation which increases the physiological effects of fat in regards to the increased release of satiety hormones, and in turn increases sensitivity to fatty acids and possibly reduces fat intake. However, due to the regular turnover of taste cells (approximately every 10-14 days) (Farbman, 1980), it could be speculated that for changes in peripheral physiology to be seen, a low-fat or high-fat diet would need to be consumed for at least 10-14 days.

Research dating back to the 1950s has investigated the influence of a test meal on taste thresholds for the primary tastes (Pangborn, 1959). However, there are conflicting opinions on the matter. Pangborn (1959) reported similar findings to those found herein in that there were no significant changes in taste thresholds for sucrose, NaCl, citric acid and caffeine pre- and post-meal (Pangborn, 1959). These results were found in a subject group of eight and these subjects were trained panellists. Although the present study did not test oral fatty acid thresholds prior to the test meal, the fact that there were no significant differences in thresholds after each test meal suggests that the macronutrient composition of the meal did not affect oral fatty acid detection thresholds. Similarly, Pasquet et al. (2006) concluded that no differences were seen in taste thresholds for sucrose, fructose, NaCl, quinine sulphate, PROP and liquorice after a meal (*ad libitum* consumption of a standard dish of sweetened cream) was consumed (Pasquet, et al., 2006), thus backing up the

findings of the present study. In contrast, other studies have found that taste sensitivity to sweet and salty substances were lower before a meal compared to after a meal, however no differences were found for bitter substances (Zverev, 2004). The authors postulate that the differences pre- and post-meal may be due to differential biological roles of sweet, salty and bitter tastants. Differences between studies may reflect the design of the studies with some studies testing thresholds before and after the test meal, while others only testing thresholds after the meal. Differences in the composition of the meal consumed may explain contrasting results, however this is difficult to determine as some studies state exactly what subjects consumed (*e.g.,* sweetened cream) whilst others describe the meal as a 'standard lunch in the university cafeteria' (Pasquet, et al., 2006; Zverev, 2004). Suchecka et al. (2011) investigated this hypothesis and measured taste thresholds for sweet, salty and sour prior to consumption of a high-carbohydrate meal and post-meal (Suchecka, et al., 2011). It must be noted that while detection thresholds are academically interesting, some have reported that thresholds have little effect on food sensations and intake, for example, it has been suggested that thresholds cannot predict intake of salt or alcohol (Hayes & Keast, 2011; Keast & Roper, 2007). Suchecka et al. (2011) also reported that there was a sex-dependent effect on taste sensitivity. Sweet taste perception was not significantly different in females, however a significant decrease was found for men. Likewise, a significant increase was found for sour taste in women, but not men. No differences were reported for salty taste pre- and post-meal (Suchecka, et al., 2011). This study suggests that potentially there is an effect on taste thresholds when a specific macronutrient rich meal is consumed, nevertheless these results are not conclusive. These results are different to those reported in the

present study, as no significant changes were found in oral fatty acid sensitivity after a high-fat meal and there were no differences found between males and females, however the present study did not test thresholds prior to the test meal. Differences between studies may be due to differences in study design and perhaps the present study may have found similar results if thresholds were tested before and after the test meal. This would seem logical as previous research has found that oral fatty acid sensitivity can be modulated over a four week period (Stewart & Keast, 2012). Therefore, it seems that much more research is required in this area to determine a definitive answer.

Until recently, the reliability of oral fatty acid thresholds was unknown, nevertheless emerging evidence now exists which shows the test-retest reliability of the fatty acid threshold measure (Newman & Keast, 2013; Tucker & Mattes, 2013). The present study contributes further to these findings with strong correlations found both across testing days for the first threshold measurement and within testing days. However, the second across day measurements were not strongly correlated. Interestingly, although there were no significant differences found between sessions one and two on each testing day, the thresholds for session one were higher than session two which suggests that perhaps there was a learning effect occurring each day, or a blunting effect from the meal. This is not a novel finding as Tucker et al. (2013) found variability in measurement between sessions and proposed that subjects need to be tested up to seven times over numerous days before a stable threshold measure can be found (Tucker & Mattes, 2013). However in the present study after the high-fat meal, 10 out of the 31 subject's thresholds were higher than their first threshold,

after the low-fat meal, 8 subject's thresholds were higher and after the balanced meal 10 subject's thresholds were higher than their first threshold. Therefore, a learning effect may not have occurred and this is a similar finding to previous work whereby reliability over numerous sessions was strong (Newman & Keast, 2013). In addition, this study found high inter-individual variation between subjects and low intra-individual variability within subjects which is in line with previous findings (Newman & Keast, 2013; Running, et al., 2013). Fatty acids do not act as a traditional taste stimuli and there is no apparent lexicon for the taste of fat, therefore it can be quite difficult for subjects to identify fatty acids as taste stimuli.

Interestingly, some subjects were unable to obtain a threshold measure at concentrations used in previous research (0.02, 0.06, 1.4, 2.0, 2.8, 3.8, 5.0, 6.4, 8.0, 9.8, 12 mM), thus an additional concentration (20 mM) was used to ensure a threshold for all subjects was established. Due to the acidic moiety of fatty acids acting as an irritant, a concentration of 20 mM was chosen as other non-taste cues, including oral irritation, may impact on detection threshold testing at higher concentrations and it is not yet clear how to differentiate between irritancy thresholds and 'taste' thresholds (Chale-Rush, et al., 2007b). The addition of this concentration allowed all subjects to establish an oral fatty acid detection threshold.

The potential relationship between oral fatty acid sensitivity and BMI remains contentious. The present study found no relationship between oral fatty acid sensitivity and BMI and no significant differences in thresholds when subjects were grouped as lean and obese, however there is conflicting evidence with some studies

finding inverse associations (Stewart, et al., 2010; Stewart, Newman, et al., 2011; Stewart, Seimon, et al., 2011) and others finding no associations at all (Kamphuis, Saris, et al., 2003; Mattes, 2009d, 2011b; Stewart & Keast, 2012). Studies that found an association used a brief screening procedure as opposed to a complete detection threshold procedure which may account for these findings (Stewart, et al., 2010; Stewart, Newman, et al., 2011). In addition, subjects in these studies were only measured once and as emerging evidence indicates more than one measure is needed for accuracy in measurement. The fact that majority of subjects were classified in the healthy weight range may account for the fact that no associations between oral fatty acid sensitivity and BMI were found in this study.

5.7 Conclusions

The present study has provided novel data in regards to the effect that a high-fat meal may have on oral fatty acid sensitivity. This study provides evidence that the macronutrient composition of the meal immediately prior to threshold testing does not influence an individual's oral sensitivity to fatty acids. However, it is important to note that only one type of food has been tested, thus future research using different types of foods which have undergone different cooking methods and contain different ingredients are needed before a definitive statement can be made. In addition, this study provided confirmatory evidence for the test-retest reliability of oral fatty acid thresholds. It is suspected that long term changes (a minimum of four weeks) in dietary behaviour (reduction in fat consumption) would be needed before changes in oral fatty acid sensitivity would be seen.

5.8 Future directions

In furthering the research into oral fatty acid sensitivity, the next step would be to examine oral fatty acid thresholds immediately before and after a meal. Exploration into why the second threshold measure was lower than the first in the present study is also an area which may warrant further investigation. In addition, evaluation of environmental and genetic factors which may predispose an individual to attenuated sensitivity to fatty acids both in the oral cavity and GI tract requires investigation. As well as determining what time course is needed to see these changes and also potential changes in preference, as a long-term change in preference will ultimately lead to long-term changes to dietary consumption.

CHAPTER SIX

Study 3

The effect of dietary fat consumption and weight loss on oral fatty acid sensitivity

6.1 Introduction

Obesity is a worldwide epidemic that causes many deleterious health conditions including cardiovascular disease, type-2 diabetes, metabolic syndrome and cancer (World Health Organisation, 2000). Myriad factors are responsible for the obesity epidemic including environmental, physiological, cultural, socioeconomic and genetic factors (World Health Organisation, 2000). Environmental influences are of particular interest as the many changes in food habits and production over the past century may have aided in the creation of an obesogenic food environment (Cordain, et al., 2005). Contributing environmental influences directly related to food and associated with weight gain and obesity include; high energy density, larger portion size and high appetitive value (Blundell, et al., 2005). Excess fat consumption is a major cause of excess energy intake and is thus a key contributor to weight gain. Overconsumption of fat may be due to many factors including preference, appetite, satiety and availability (Blundell, Lawton, Cotton, & Macdiarmid, 1996). However, not everyone is susceptible to weight gain and there is a multitude of proposed explanations (Blundell, et al., 2005). One view suggests that fat intake may be poorly regulated in the obese population, such that the obese may not be able to control their consumption of dietary fats and therefore consume excess fat, with evidence now

indicating that dysfunctional detection of fats throughout the alimentary canal may contribute to this irregularity (Stewart, Seimon, et al., 2011).

Detection of the breakdown products of fats, fatty acids, occurs during ingestion and digestion in the oral cavity and gastrointestinal (GI) tract by putative fatty acid receptors including G-protein coupled receptors (GPCR120, GPCR40, GPCR43), Cluster of Differentiation 36 (CD36) and Delayed Rectifying Potassium (DRK) channels (Bachmanov & Beauchamp, 2007). Free fatty acids are detected throughout the alimentary canal, specifically the gustatory system where they contribute to oral chemoreception and the cephalic response, and the GI system where they influence gut motility and the hormonal satiety cascade which promotes development of satiety. In obese individuals, oral and GI detection of fatty acids is attenuated, possibly predisposing the individual to high intakes of fatty foods, and potentially a greater energy intake (Stewart, Seimon, et al., 2011).

Oral perception of free fatty acids within the oral cavity has been described by a wealth of recent evidence (Chale-Rush, et al., 2007a; Gilbertson, 1998; Mattes, 2001b, 2009a, 2009c; Stewart, et al., 2010; Stewart, Newman, et al., 2011). Several studies that controlled for non-taste cues and olfaction have found that humans can detect a range of different fatty acids including oleic acid (C18:1), linoleic acid (C18:2), stearic acid (C18:0), lauric acid (C12:0) and caproic acid (C6:0) (Mattes, 2009a, 2009c, 2009d; Stewart, et al., 2010). The reliability and reproducibility of fatty acid detection measures has been reported (Newman & Keast, 2013).

Fatty acid sensitivity throughout the oral cavity and GI tract has also been strongly associated with overweight and obesity (Little & Feinle-Bisset, 2011). During digestion, fatty acids have a potent effect on the release of satiety hormones cholecystokinin (CCK), peptide YY (PYY) and glucagon-like peptide-1 (GLP-1). These hormones act at central and peripheral locations to suppress food intake and appetite (Feltrin, et al., 2004). The ability to detect fats in the GI tract in obese individuals has been found to be dysfunctional, suggesting that an attenuated sensitivity to fats results in a diminished release of satiety hormones, resulting in excess consumption of energy and increased BMI (Little & Feinle-Bisset, 2011). Recent research investigating analogous fatty acid sensitivity in the oral cavity and GI tract, found that following an intra-duodenal infusion of C18:1, obese subjects experienced an attenuated hormonal response and consumed more energy than lean individuals (Stewart, Seimon, et al., 2011). In addition, oral fatty acid sensitivity was lower in obese participants compared to lean participants, highlighting the link between fat detection in the oral cavity and GI tract (Stewart, Seimon, et al., 2011).

Both animal and human models investigating oral fatty acid sensitivity have found an inverse relationship between oral sensitivity to fatty acids and fat consumption, which predisposes insensitive animals to excessive fat consumption and obesity (Gilbertson, et al., 1998; Stewart, et al., 2010; Stewart & Keast, 2012; Stewart, Newman, et al., 2011). Similar findings in humans suggest that oral fatty acid sensitivity could contribute to obesity, with subjects hyposensitive to fatty acids having higher BMIs than hyposensitive subjects (Kamphuis, Lejeune, Saris, & Westerterp-Plantenga, 2003). Additionally, Stewart et al. (2011) found a relationship

between fatty acid sensitivity, food intake and food behaviours, with hyposensitive individuals consuming more high-fat dairy products, fatty red meats and fatty spreads compared to hypersensitive individuals (Stewart, Newman, et al., 2011). Hypersensitive subjects also reported more readily engaging in behaviours to reduce their fat intake including trimming fat from meat and avoiding saturated fats and were more likely to follow a low-fat and low-sugar diet (Stewart, Newman, et al., 2011). Importantly, habitual consumption of a high-fat diet may be linked to oral hyposensitivity to fatty acids, with Stewart el al. (2012) finding that consumption of a low-fat diet by lean and overweight/ obese individuals increased oral sensitivity to fatty acids in both groups, however consumption of a high-fat diet decreased oral sensitivity to fatty acids, but only in the lean group (Stewart & Keast, 2012). These results suggest habituation to a high-fat diet has occurred in the obese individuals, potentially causing an adapted reduction in the physiological and psychological effects of fat that may result in a reduction in oral sensitivity to fats and possibly promote obesity. Accordingly, individuals who consume less dietary fat and engage in fat reducing behaviours may be decreasing their susceptibility to adaptation to a high-fat diet, and therefore these behaviours may influence an individual's oral sensitivity to fatty acids or *vice versa*. However, the direction of this relationship and the underlying mechanisms are unknown.

To date, there has been no research investigating the potential link between oral fatty acid sensitivity and weight loss via dietary changes. The current obesity epidemic reflects an imbalance of energy intake and expenditure. However, not everyone is susceptible to diet induced weight gain and identifying potential characteristics such

as appetite control and food motivation which may be causal influences, is an important step in determining how overweight and obesity could be treated and prevented (Blundell, et al., 2005). Low-fat diets have previously been used as a successful tool for weight loss in the overweight/ obese group, as have energy restriction (portion control) diets (Bray, et al., 2004), with weight loss averaging 1.6 g/ day for each 1% decrease in fat intake (Bray, et al., 2004). Hypothetically, lean or overweight/ obese subjects following a low-fat diet would experience weight loss and demonstrate increased sensitivity to fatty acids throughout the alimentary canal. The increased sensitivity to dietary fat may also result in appetitive changes, whereby smaller amounts of fat would be required to elicit acceptable post-ingestive effects and satiety responses, which in turn help to maintain long-term fat and energy intake reduction. Recent unpublished research from our laboratory supports this proposal, in that fat insensitive subjects were less satiated by a high-fat meal and consumed more energy at a buffet meal than fat sensitive subjects (Keast, et al., 2013). These findings further suggest that attenuated fatty acid sensitivity throughout the alimentary canal is linked to appetitive processes, including satiety, as well as excess fat consumption and weight gain. Currently, however, it is unclear what determines or influences sensitivity to fats, the relationship with obesity and whether it can be affected by weight loss.

6.2 Aim and hypotheses

6.2.1 Aims

To determine the effect of a low-fat weight loss diet (25% total dietary fat) and a portion control weight loss diet (33% total dietary fat) on oral fatty acid detection thresholds, the ability to detect the fat content of food and preference for low-fat foods.

6.2.2 Hypotheses

It is hypothesised that consumption of a low-fat diet for six weeks will decrease an individual's fat intake (both grams and percentage), increase oral fatty acid sensitivity, increase the ability to detect the fat content of foods and increase preference for low-fat foods compared to consumption of a portion control diet, in overweight/ obese subjects.

6.3 Subjects, materials and methods

6.3.1 Subjects

A power calculation was conducted to determine an appropriate sample size for the study. Data were used from a previous Deakin University study (Stewart & Keast, 2012) involving a dietary intervention and measurement of oral fatty acid sensitivity. In this study, consumption of a low-fat diet for four weeks trended to decrease taste thresholds for C18:1 (baseline: 4.8 ± 4.4 [range 0.06 – 12] mM; end of intervention: 2.6 ± 2.8 [range: 1 – 12] mM) (Stewart & Keast, 2012). Thus, for the present study we expected to detect a threshold difference of 2.2 mM C18:1 between the two time points (baseline and week six). The standard deviation value for fatty acid detection

thresholds (3.1 mM C18:1, $\sigma = 3.1$) was also based on the study by Stewart et al. (2012). α, the z-score probability of a Type I error, was set at 1.96 for 95% confidence, and β, the z-score probability of a Type II error, was set at 1.64 for 90% confidence. Therefore, 26 people per dietary group were required for the study to have adequate power. Due to the 15% attrition rate in the Stewart et al. (2012) study, we conservatively aimed to recruit at least 25% additional participants per dietary group (resulting in at least 33 participants per group).

Subjects were recruited from the suburbs surrounding Deakin University, Burwood, Victoria, Australia. To be eligible for the study, subjects had to meet the following criteria: BMI > 25 kg/m^2, non-smoker and 18-75 years of age. Subjects were excluded if they were pregnant or breast feeding or had a medical condition that affected their taste or weight loss ability. This study was approved by the Deakin University Human Research Ethics Committee and registered with the Australian New Zealand Clinical Trials Registry (ANZCTR trial number: ACTRN12611000679987) as a clinical trial. Written informed consent was obtained from all subjects prior to their first testing session.

6.3.2 Study outline

This study was a randomised dietary intervention study involving subjects following one of two diets: 1) a low-fat diet (< 25% total energy from fat) or 2) a portion control diet (reduction of energy intake by 25%). All subjects were required to attend two laboratory sessions throughout the six week period, once at baseline and once at six weeks. At each session, subjects completed a number of tests which included:

detection threshold tests for C18:1, sucrose and sodium (NaCl) using ascending forced choice triangle tests; a fat ranking task using custard samples with varying fat contents; preference ratings for low-fat and regular-fat foods; and anthropometric measures including height, weight and waist and hip circumference. Subjects were also required to complete a one-day diet record on a weekly basis to establish habitual intake and compliance, a food frequency questionnaire to validate habitual intake, and a Community Healthy Activities Model Program for Seniors (CHAMPS) physical activity questionnaire at baseline and week six.

6.3.3 Low-fat diet

This diet was designed to reduce the consumption of full fat dairy products, fatty red meats, baked products, high-fat spreads and overall kilojoule intake and aimed to have subjects consuming 25% total energy from fat each day (outlined in more detail in **Chapter 3**). Dietary counselling was overseen by the coordinating dietitian (S.T.) and provided by trained research staff. Subjects were given a 30-45 minute face-to-face counselling session at baseline and a booklet which contained all of the information needed to follow the diet **(Appendix I)**. Subjects were instructed to consume at least 5 portions of vegetables and 2 portions of fruit per day, as well as 3 portions of low-fat dairy per day, 1-2 portions of meat and fish per day, a maximum of 3 portions of fats per day and a maximum of 5 portions of breads and cereals per day (portion sizes are outlined in **Chapter 3**). A list of snack food ideas, tips for reducing kilojoule intake from day to day, tips for dining outside of the home and how to read food labels were also provided. As alternatives to high-fat spreads and snack foods, subjects were given low fat margarine, four packets of low fat crackers

and one packet of popcorn kernels to assist with compliance. Subjects were also contacted by phone on a weekly basis to answer any questions that may have arisen and to keep subjects motivated and accountable.

6.3.4 Portion control diet

The portion control diet was designed to decrease subjects' energy intake by 25%, but consume the same percentage of energy from fat (33% total fat) **(Table 6.1)** and was based on the Australian government campaign, 'Swap it, don't stop it' (2012). Subjects were instructed to reduce their usual food intake **(Chapter 3)**. Subjects also received a 30-minute one-on-one counselling session with trained research staff to explain how to reduce their energy intake by 25% (*i.e.,* how to reduce the amount of food on their plate at dinner time, but keep the same proportion of each food component). Participants were given a list of instructions to help reduce portions: 1) use a smaller plate to make small amounts of food seem larger; 2) eat mindfully by taking time to chew food properly and eat slowly (Smit, et al., 2011); 3) avoid distractions such as watching television or reading, which can distract you from noticing when you are full; 4) follow the 80 percent rule to stop eating before you are completely full ("Sizing up food portions," 2010) **(Appendix J)**. Subjects were given a small, appetiser sized plate (23 cm) for all meals, as studies indicate subjects are eat less when using smaller plates (Laddu, et al., 2011; Pedersen, et al., 2007; "Sizing up food portions," 2010). As with the low-fat diet group, weekly calls were made to those following the portion control diet to answer any underlying questions and to keep subjects motivated.

Table 6.1: The intended macronutrient profile of the low-fat and portion control diet groups

	Low-fat group*	Portion control group**
Total energy intake (kJ)	6760 kJ	6827 kJ
Percentage energy from fat	25%	33%
Percentage energy from carbohydrates	53%	45%
Percentage energy from proteins	18%	18%
Percentage energy from alcohol	4%	4%

* Based on meal plan developed by researchers

** Based on a 25% reduction of the average Australian intake for adult males and females aged from 25-64 years (National Health and Medical Research Council, 2006)

6.3.5 Anthropometry

Body weight (kg) was measured at baseline and week six with light, loose clothing and shoes removed. Height (m) was measured without shoes at baseline and week six using a portable stadiometer. Body mass index (BMI; weight (kg)/ height (m^2)) was then calculated from these values and subjects were categorised as overweight ≥ 24.9 ≤ 29.9 kg/ m^2, or obese ≥ 30 kg/ m^2 (Harris, et al., 2008). Each subject's waist and hip circumference was also measured at baseline and week six according to the methods described by Gibson (2005).

6.3.6 Food consumption and dietary compliance

Each subject completed a one-day food record at baseline and weeks two, three, four and six, reporting all foods and drinks consumed in household measures. Subjects were asked to, where possible, weigh the foods they consumed (subjects used their own scales), use standard metric measuring cups, or common serving sizes **(Chapter 3)**. The one-day food records were analysed using the AUSNUT 2007 food composition database and FoodWorks 2009 software (Xyris software, Highgate Hill, Queensland, Australia). From these data, the mean energy intake (kJ), macronutrient distribution (% energy from fat, protein and carbohydrate, and grams of fat, protein and carbohydrate), type of fat (grams and % of monounsaturated, polyunsaturated or saturated), amount of sodium (mg), and amount of alcohol (g) consumed were calculated.

6.3.7 Diet questionnaires

Subjects were also required to complete a food frequency questionnaire **(Appendix G)** that recorded how frequently they consumed common foods identified in the 1995 Australian National Nutrition Survey (Australian Bureau of Statistics, 1995). In total, 120 food items were assessed including foods from these categories: dairy products; bread and cereals; meat, fish and eggs; other offal; sweets, baked goods and snacks; dressings; non-dairy beverages; vegetables; fruits; and vitamin and mineral supplements. Subjects were asked how often they consumed these items and could answer on a scale ranging from ‘never or less than once a month’ to ‘six or more times per day’ (more detail can be found in **Chapter 3**). Each frequency category was converted into a daily equivalent value, for example, ‘never, or less

than once a month' = 0.02, 'one to three times per month' = 0.07, 'once per week' = 0.1, 'two to four times per week' = 0.4, 'five to six times per week' = 0.8, 'once per day' = 1.0, 'two to three times per day' = 2.5, 'four to five times per day' = 4.5 and 'six plus times per day' = 6. The foods were then categorised into the following specific food groups red meat; poultry; processed meat; organ meat; fish, seafood; refined grains; whole grains; eggs; high-fat dairy; low-fat dairy; soy; liquor spirits; wine; beer; hot drinks; fruit; leafy vegetables; cruciferous vegetables; other vegetables; tomatoes; peas and legumes; nuts; potatoes; high-fat take away foods; fried foods; snacks; desserts and biscuits; chocolate and sweets; sugar sweetened beverages; low-energy beverages; condiments; and salad dressing.

6.3.8 Physical activity

As part of a healthy lifestyle change, all subjects were required to participate in a minimum of 30 minutes of moderate intensity physical activity on all or most days of the week (Sims, et al., 2010). Information was provided to subjects on how to calculate their maximum heart rate (220 – age (years)) and subjects were asked to workout at a level where their heart rate was 60-70% of their maximum heart rate. This level was considered moderate intensity and subjects were advised to exercise at this level for the full 30 minutes. The amount of exercise completed was measured at baseline and week six using the CHAMPS questionnaire (Stewart, et al., 2001) and this information was used to calculate the average hours per week of physical activity across the intervention period for each subject **(Appendix K)**.

6.3.9 C18:1 solutions

Food grade C18:1 was stored in nitrogen below 4°C. C18:1 was added to long-life non-fat milk at varying concentrations (0.02, 0.06, 1, 1.4, 2, 2.8, 3.8, 5, 6.4, 8, 9.8, 12 and 20 mM) **(Chapter 3)**. To minimise textural differences imparted by C18:1, all samples were mixed with 5% gum acacia and liquid paraffin. To prevent oxidation of C18:1, 0.01% w/v ethylenediaminetetraacetic acid (EDTA) was added to all samples. A stable emulsion was created by first mixing the milk and gum together, then adding it to the paraffin and fatty acid mixture. Samples were homogenised at 12 000 rpm for 30 sec/ 100 ml. All samples were freshly prepared each day of testing. Control samples were prepared in the same way, with the fatty acid omitted.

6.3.10 C18:1 detection thresholds

Oral fatty acid sensitivity was determined via detection thresholds, using triangle tests with ascending forced choice methodology. Subjects were presented with three samples, two control and one containing C18:1 in an ascending concentration order from the lowest (0.02 mM) to the highest (20 mM) concentration. (Newman & Keast, 2013; Stewart, et al., 2010). Detection thresholds for each fatty acid were defined as the concentration at which the subject correctly picked the odd sample in three consecutive sample sets. To minimise confounding factors from non-taste sensory inputs, all testing was performed under red lighting and nose clips were worn at all times. Subjects were also instructed to taste the samples and then spit them out. Subjects were required to refrain from eating and drinking (water was allowed) one hour prior to testing.

6.3.11 Sucrose and NaCl solutions and detection thresholds

Solutions were prepared in accordance with the International Organisation for Standardisation **(Chapter 3)**. The eight different concentrations of each tastant (sucrose and NaCl) were then prepared according to concentrations in **Table 3.1**. Distilled water was used as the control sample in threshold determinations. Testing for sucrose and NaCl thresholds was conducted in the same way that C18:1 thresholds were measured (triangle tests with ascending forced choice methodology).

6.3.12 Fat ranking task

For this task, subjects were provided with custard samples made with (0, 2, 6 or 10%) canola oil **(Chapter 3)**. Custard was made from 20 g custard powder, 12 g sugar and 500 ml non-fat milk. The custard was separated into four batches and canola oil was added to three of the batches to create custards containing 2%, 6% or 10% fat. No oil was added to one custard batch to create the 0% sample. All batches of the custard were sensory matched with paraffin oil to contain 10% oil. Subjects were asked to rank the four custard samples in order of perceived fattiness. All subjects received a score out of five for this task **(Table 3.2)**.

6.3.13 Hedonic ratings

Subjects completed a preference test with three sets of regular-fat (RF) and low-fat (LF) foods. Subjects were given a variety of different foods including cream cheese (RF: Philadelphia Spreadable Cream Cheese Original; LF: Philadelphia Spreadable Cream Cheese Extra Light, Kraft Foods Limited, South Wharf, Victoria, Australia), vanilla yoghurt (RF: Yoplait Creamy Original Vanilla yoghurt; LF: Yoplait Creamy

Lite Vanilla yoghurt, National Foods, Docklands, Victoria, Australia) and chocolate mousse (RF: Nestle Chocolate Mousse; LF: Nestle Diet Chocolate Mousse, Nestle, Fonterra Brands, Auckland, New Zealand) **(Chapter 3)**. Liking was measured on a 9-point hedonic scale ranging from 'dislike extremely' to 'like extremely'. Subjects were asked to taste both samples and rate their liking of each one.

6.3.14 Tongue papillae photography

Before tongue photographs were taken, subjects were asked to rinse their mouths thoroughly with deionised water. They were then asked to sit with their elbows on the photography bench and their hands held together in a 'V' formation, supporting their chin and keeping their head still **(Chapter 3)**. Subjects were asked to protrude their tongue and use their lips to keep it steady, and the camera was then lined up with the participant's tongue. The tongue was dried using filter paper and diluted blue food dye was applied to the left of the midline of the tongue using a cotton bud. The tongue was again dried with filter paper to remove excess dye, and a square piece of filter paper (10 mm x 10 mm) with a 6 mm diameter hole in the centre was placed on the tip of the tongue over the dyed area. Three macro photographs were then taken of the subject's tongue. The photos were analysed using Adobe Photoshop version CS5.1 (Adobe Systems Inc., San Jose, CA, USA) to count the fungiform papillae. The fungiform papillae were identified as the structures that were stained in a very light shade of blue. When papillae were hard to distinguish or difficult to confirm, the zoom function in Adobe Photoshop was used to magnify the image **(Chapter 3)**.

6.3.15 6-*n*-propylthiouracil (PROP) sensitivity

PROP paper strips were prepared according to methods outlined in **Chapter 3.** Subjects were presented with a piece of filter paper which contained the PROP solution and asked to place it on the centre of their tongue until it was soaked with saliva. Following this, subjects were asked to rate the perceived intensity of the bitterness of the PROP using a general Labelled Magnitude Scale (gLMS). The gLMS has descriptors placed at varying positions which range from 'strongest imaginable' to 'barely detectable' **(Appendix C)**. Subjects were briefly trained in the use of this scale and told that 'strongest imaginable' sensation was the pain caused if a dentist drilled a hole in your tooth without any anesthetic, and 'barely detectable' was tastes like paper.

6.4 Statistical analysis

Data were analysed using SPSS version 21 (SPSS Inc., Chicago, IL, USA). Repeated-measures analysis of variance (ANOVA) was used to analyse changes in C18:1, sucrose and NaCl oral detection thresholds; hedonic ratings for regular-fat and low-fat foods; anthropometric measurements; tongue papillae numbers and dietary intake from baseline to week six with time-point as within-subject factors and dietary intervention (low fat or portion control) as between-subject factors. Wilcoxon Signed-Rank tests were used to detect differences in fat ranking scores from baseline to week six, and Mann-Whitney U tests were used for between-group analyses. Bonferroni corrected paired t-tests were used to establish differences in answers to dietary questionnaires from baseline to week six. All values are stated as mean ± SEM.

6.5 Results

6.5.1 Subjects

Of the 105 overweight/ obese men and women who attended a screening visit, 8 were ineligible due to health concerns (5 subjects) or food allergies (3 subjects). Of the 97 who were eligible for the study, 25 decided not to participate prior to commencement of testing (6 – work commitments, 6 – health problems, 3 – personal reasons, 10 – no reason). Seventy-two subjects were randomised into one of the two dietary interventions (36 in the low-fat (LF) group and 36 in the portion control (PC) group). After randomisation, a further eight subjects withdrew due to work commitments and compliance issues (LF – 5, PC – 3). Visual inspection of box plots identified a further 11 subjects as being three standard deviations away from the mean for total energy (kJ), total fat (g) and percentage energy from fat, and were excluded from final analysis. These subjects were removed as their dietary data was viewed as non-compliant and inaccurate due to the fact that they had consumed far less kilojoules than required for their basal metabolic rate (BMR). For example, some subjects had reported consuming 1500 kJ for a whole day, therefore these subjects were removed, which normal practise in dietary studies. In total, 53 subjects (LF – 26, PC – 27) completed the study. Participants were on average middle aged and overweight or obese (BMI $\geq$ 25 kg/m^2), with waist-hip ratios $\geq$ 0.9 cm which puts them at a substantially increased risk of metabolic complications (Nishida, Ko, & Kumanyika, 2010). There were no significant differences between the groups at baseline for any of the measures **(Table 6.2)**.

Table 6.2: Comparison of subjects' demographic, anthropometric and oral detection threshold characteristics at baseline and week six for the total sample and diet group

	All (*n* = 53)	LF (*n* = 26)	PC (*n* = 27)	*P* value
Sex (M/F)	17/36	8/18	9/18	0.842[a]
Age (years)	56.5 ± 1.9	56.7 ± 2.2	56.3 ± 3.2	0.904[a]
Weight (kg)				
Baseline	83.5 ± 2.4	86.9 ± 3.7	81.7 ± 3.0	0.374[a]
Week 6	81.9 ± 2.4	84.4 ± 3.7	79.5 ± 3.0	< **0.001**[b]
Change	-1.9 ± 0.3	-2.5 ± 0.4	-2.2 ± 0.3	
BMI (kg/m^2)				
Baseline	31.0 ± 0.7	32.7 ± 1.2	29.9 ± 0.8	0.105[a]
Week 6	30.6 ± 0.7	31.8 ± 1.1	29.4 ± 0.8	< **0.001**[b]
Change	-0.4 ± 0.1	-2.9 ± 0.2	-2.4 ± 0.1	
Waist-Hip ratio (cm)				
Baseline	0.9 ± 0.01	0.9 ± 0.01	0.9 ± 0.02	0.069[a]
Week 6	0.7 ± 0.1	0.7 ± 0.2	0.7 ± 0.2	0.032[b]
Change	-0.2 ± 0.1	-0.2 ± 0.2	-0.2 ± 0.1	
C18:1 detection threshold (mM)				
Baseline	8.0 ± 0.2	9.8 ± 0.2	6.4 ± 0.3	0.654[a]
Week 6	5.1 ± 0.2	6.4 ± 0.3	3.7 ± 0.2	**0.014**[b]
Change	-2.9 ± 0.2	-3.1 ± 0.3	-2.8 ± 0.4	
Sucrose detection threshold (mM)				
Baseline	6.3 ± 0.7	6.0 ± 0.9	6.5 ± 1.1	0.627[a]
Week 6	5.0 ± 0.7	5.1 ± 0.8	4.9 ± 1.1	0.227[b]
Change	-1.3 ± 1.0	-0.9 ± 1.3	-1.6 ± 1.6	
NaCl detection threshold (mM)				
Baseline	0.4 ± 0.1	0.3 ± 0.04	0.4 ± 0.11	0.174[a]
Week 6	0.6 ± 0.5	0.1 ± 0.1	1.2 ± 0.9	0.558[b]
Change	0.2 ± 0.5	-0.2 ± 0.1	0.8 ± 0.9	
Moderate intensity physical activity (hours/ week)				
Baseline	13.0 ± 1.0	11.4 ± 1.1	14.8 ± 1.8	0.277[a]
Week 6	12.8 ± 0.8	11.9 ± 1.2	13.9 ± 1.1	0.815[b]
Change	-0.2 ± 0.8	0.5 ± 0.9	-0.9 ± 1.3	

All values are presented as Mean ± SEM. BMI, body mass index; LF, low-fat group; PC, portion control group

[a] indicates a *P* value for a difference at baseline between LF and PC using independent samples t-test

[b] indicates a *P* value for a main effect of time using repeated measures ANOVA

6.5.2 Anthropometry

Weight

Consumption of both the low-fat and portion control diets resulted in a significant reduction in weight from baseline to week six (main effect of time: Wilks' Lambda = 0.479, $F(1, 47) = 51.2$, $P < 0.001$) and a significant group by time interaction (time*group interaction: Wilks' Lambda = 0.900, $F(1, 47) = 5.22$, $P = 0.027$). No significant differences between groups were observed (main effect of group: $F(1, 47) = 1.29$, $P = 0.262$), meaning that there was a mean decrease in weight during the intervention and there was no difference in weight loss between diets. Nine out of the 53 subjects did not lose weight, but the amount of weight gained was less than 1 kg. However, the portion control group weighed less at baseline than the low-fat group, which may explain the significant interaction effect. The weight of the two groups did not differ at week six ($P = 0.391$) **(Table 6.2)**.

BMI

Consumption of both diets resulted in a significant decrease in BMI from baseline to week six (main effect of time: Wilks' Lambda = 0.528, $F(1, 47) = 42.1$, $P < 0.001$) and a significant group by time interaction was observed (time*group interaction: Wilks' Lambda = 0.894, $F(1, 47) = 5.58$, $P = 0.022$). However, there were no between group effects (main effect of group: $F(1, 47) = 3.46$, $P = 0.069$) **(Figure 6.1 A)**, meaning that there was a mean reduction in BMI regardless of their allocated diet.

Waist and hip circumference and waist-hip ratio

Waist circumference was significantly reduced from baseline to week six (main effect of time: Wilks' Lambda = 0.566, $F(1, 47) = 36.0$, $P < 0.001$), as was hip circumference in both groups (main effect of time: Wilks' Lambda = 0.521, $F(1, 47) = 43.3$, $P < 0.001$), however there were no significant changes between groups (main effect of group: $F(1, 47) = 0.304$, $P = 0.584$; $F(1, 47) = 1.37$, $P = 0.247$; waist and hip circumference, respectively) or interactions between time and group (time*group interaction: Wilks' Lambda = 1.00, $F(1, 47) = 0.014$, $P = 0.906$; Wilks' Lambda = 0.973, $F(1, 47) = 1.29$, $P = 0.263$; waist and hip circumference, respectively). In comparison to baseline, waist-hip ratio at week six was significantly reduced (main effect of time: Wilks' Lambda = 0.913, $F(1, 51) = 4.87$, $P = 0.032$), however no significant changes were seen between groups (main effect of group: $F(1, 51) = 0.007$, $P = 0.934$) and there were no interactions between time and group (time*group interaction: Wilks' Lambda = 1.00, $F(1, 51) = 0.001$, $P = 0.984$) **(Table 6.2)**, meaning that all subjects reduced their waist-hip ratio regardless of what diet they were following.

6.5.3 Dietary compliance

Average intakes of total energy (kJ), fat, saturated fat, carbohydrate and protein (g), as well as comparative (%) macronutrient distributions from the one day food records for both groups are reported in **Table 6.3**. At baseline, the two dietary groups did not differ in total energy intake or macronutrient intake **(Table 6.3)**.

Table 6.3: Mean ± SEM macronutrient intakes for the low-fat and portion control diet groups at baseline and week six

	LF (*n* = 26)	PC (*n* = 27)	*P* value
Total energy (kJ/ day)			
Baseline	7150.8 ± 384.5	7262.3 ± 500.6	0.204[a]
Week 6	6166.4 ± 209.7	5693.6 ± 250.2	**0.001**[b]
Change	-984.4 ± 373.9	-1568.6 ± 614.4	
Total fat (g/ day)			
Baseline	64.1 ± 5.2	64.4 ± 5.1	0.895[a]
Week 6	41.4 ± 2.6	47.5 ± 3.2	< **0.001**[b]
Change	-22.7 ± 5.8	-16.8 ± 6.5	
Total carbohydrates (g/ day)			
Baseline	185.8 ± 9.9	171.7 ± 16.2	0.659[a]
Week 6	182.9 ± 7.1	148.2 ± 7.4	0.190[b]
Change	-2.9 ± 10.5	-23.5 ± 17.3	
Total protein (g/ day)			
Baseline	77.8 ± 5.5	89.0 ± 5.4	0.177[a]
Week 6	75.3 ± 2.5	72.6 ± 4.1	**0.031**[b]
Change	-2.54 ± 5.3	-16.4 ± 8.4	
Percentage energy from fat (%/ day)			
Baseline	33.1 ± 1.7	32.9 ± 1.3	0.186[a]
Week 6	24.9 ± 1.04	29.2 ± 1.0	< **0.001**[b]
Change	-8.2 ± 2.0	-3.9 ± 1.5	
Percentage energy from carbohydrates (%/ day)			
Baseline	39.5 ± 1.7	34.9 ± 1.5	0.362[a]
Week 6	45.6 ± 1.3	41.6 ± 1.4	< **0.001**[b]
Change	6.1 ± 1.9	6.7 ± 1.2	
Percentage energy from protein (%/ day)			
Baseline	18.6 ± 1.0	22.6 ± 0.7	0.444[a]
Week 6	21.5 ± 0.7	21.7 ± 0.9	0.204[b]
Change	2.9 ± 1.0	-0.8 ± 1.3	

All values are presented as Mean ± SEM. LF, low-fat group; PC, portion control group; kJ, kilojoule; g, grams

[a] indicates *P* value for a difference between groups at baseline using independent samples t-tests

[b] indicates *P* value for a main effect of time using repeated measures ANOVA

Total energy (kJ) and fat (g) intake and percentage energy from fat

Between baseline and week six, both groups decreased their total energy intake (main effect of time: Wilks' Lambda = 0.762, $F(1, 42) = 13.1$, $P < 0.001$), total fat intake (g) (main effect of time: Wilks' Lambda = 0.671, $F(1, 42) = 20.6$, $P < 0.001$) and percentage of energy derived from fat (main effect of time: Wilks' Lambda = 0.676, $F(1, 42) = 21.1$, $P < 0.001$) **(Table 6.3)**. Total energy intake, fat intake and fat percentage did not differ between the two diets (main effect of group: $F(1, 42) = 0.18$, $P = 0.676$; $F(1, 42) = 0.476$, $P = 0.494$; $F(1, 42) = 2.12$, $P = 0.146$; total energy, fat, fat percentage, respectively) **(Figure 6.1 B, C, D)**. There were no time by group interactions for these changes (time*group interaction: Wilks' Lambda = 0.984, $F(1, 42) = 0.686$, $P = 0.412$; Wilks' Lambda = 0.989, $F(1, 42) = 0.454$, $P = 0.504$; total energy, fat, respectively), however there was a trend for the groups to differ in percentage energy from fat (Wilks' Lambda = 0.921, $F(1, 42) = 3.79$, $P = 0.058$).

Total carbohydrate (g) intake and percentage energy from carbohydrate

There were no significant differences between groups, changes over time or group by time interactions for total carbohydrate intake (main effect of time: Wilks' Lambda = 0.960, $F(1, 42) = 1.77$, $P = 0.190$, main effect of group: $F(1, 42) = 3.28$, $P = 0.077$, time*group interaction: Wilks' Lambda = 0.975, $F(1, 42) = 1.08$, $P = 0.306$) **(Table 6.3)**, indicating carbohydrate intake remained the same for all subjects regardless of the assigned diet. Percentage energy from carbohydrates was significantly increased in both diet groups from baseline to week six (main effect of time: Wilks' Lambda = 0.561, $F(1, 42) = 32.9$, $P < 0.001$) and this change was also

seen overall between groups (main effect of group: $F(1, 42) = 4.79$, $P = 0.034$). There were no group by time interactions (time*group interaction: Wilks' Lambda = 0.998, $F(1, 42) = 0.078$, $P = 0.782$) **(Figure 6.1 E, F)**, thus all subjects increased the percentage of carbohydrates consumed and there were no differences depending on diet type.

Total protein (g) intake and percentage energy from protein

Total protein intake significantly decreased in the low-fat diet and portion control diet groups (main effect of time: Wilks' Lambda = 0.894, $F(1, 42) = 4.96$, $P = 0.031$) **(Table 6.3)**. However, these changes did not differ significantly overall between groups (main effect of group: $F(1, 42) = 1.15$, $P = 0.290$) and there was no group by time interaction (time*group interaction: Wilks' Lambda = 0.935, $F(1, 42) = 2.91$, $P = 0.095$). Therefore, all subjects reduced their total protein intake and this did not depend on what diet was consumed. Similarly, for percentage energy from protein, there was no significant effect of time from baseline to week six (main effect of time: Wilks' Lambda = 0.962, $F(1, 42) = 1.66$, $P = 0.204$) or overall between group differences (main effect of group: $F(1, 42) = 3.39$, $P = 0.073$), however a significant interaction between time by group was observed (time*group interaction: Wilks' Lambda = 0.885, $F(1, 42) = 5.44$, $P = 0.025$) **(Figure 6.1 G, H)**. Thus, there were no differences in percentage of protein consumed between the diets, although an interaction effect may have occurred as the low-fat group had lower percentage protein consumption at baseline.

Sugar (g) and sodium (mg) intake

There were no significant differences over time or group by time interactions for sugar intake (main effect of time: Wilks' Lambda = 0.988, $F(1, 51) = 0.598$, $P = 0.443$, time*group interaction: Wilks' Lambda = 0.960, $F(1, 51) = 2.11$, $P = 0.152$). However, there was a significant difference between groups (main effect of group: $F(1, 51) = 7.58$, $P = 0.008$). There were no significant differences between groups, changes over time or group by time interactions for sodium consumption (main effect of time: Wilks' Lambda = 0.932, $F(1, 51) = 3.73$, $P = 0.059$, main effect of group: $F(1, 51) = 0.290$, $P = 0.592$, time*group interaction: Wilks' Lambda = 0.997, $F(1, 51) = 0.178$, $P = 0.674$), indicating that sodium intake remained the same for all subjects regardless of the assigned diet.

Figure 6.1: Mean ± SEM BMI and macronutrient intake in the low-fat (n = 26) and portion control (n = 27) diet groups at baseline (BL) and week six (WK 6). **A.** BMI; **B.** energy intake; **C.** total fat intake; **D.** percentage energy from fat

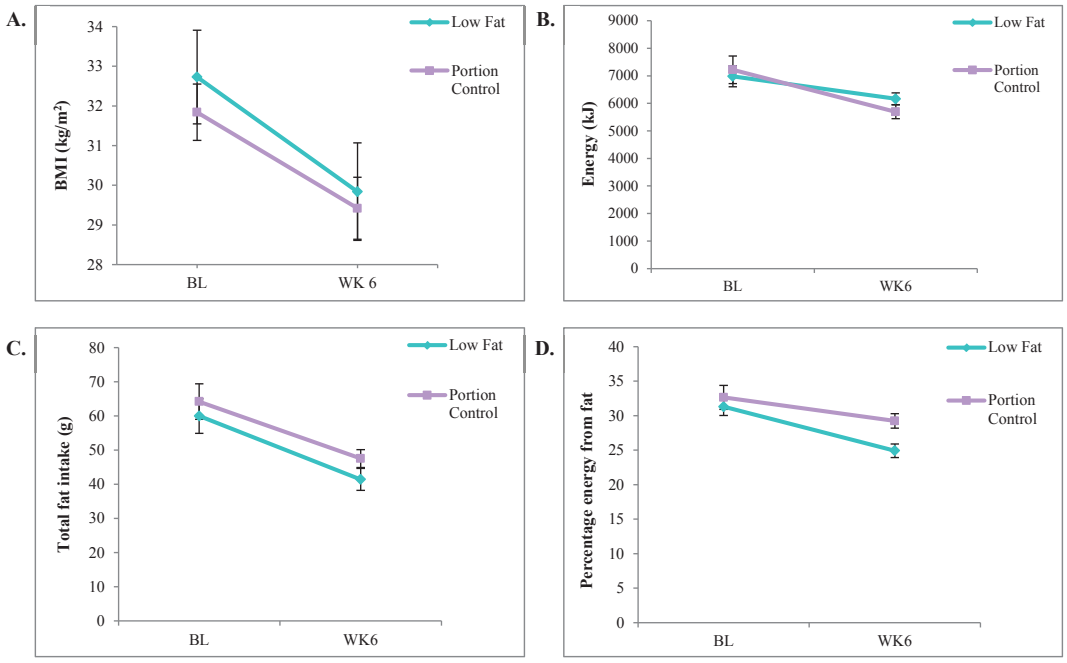
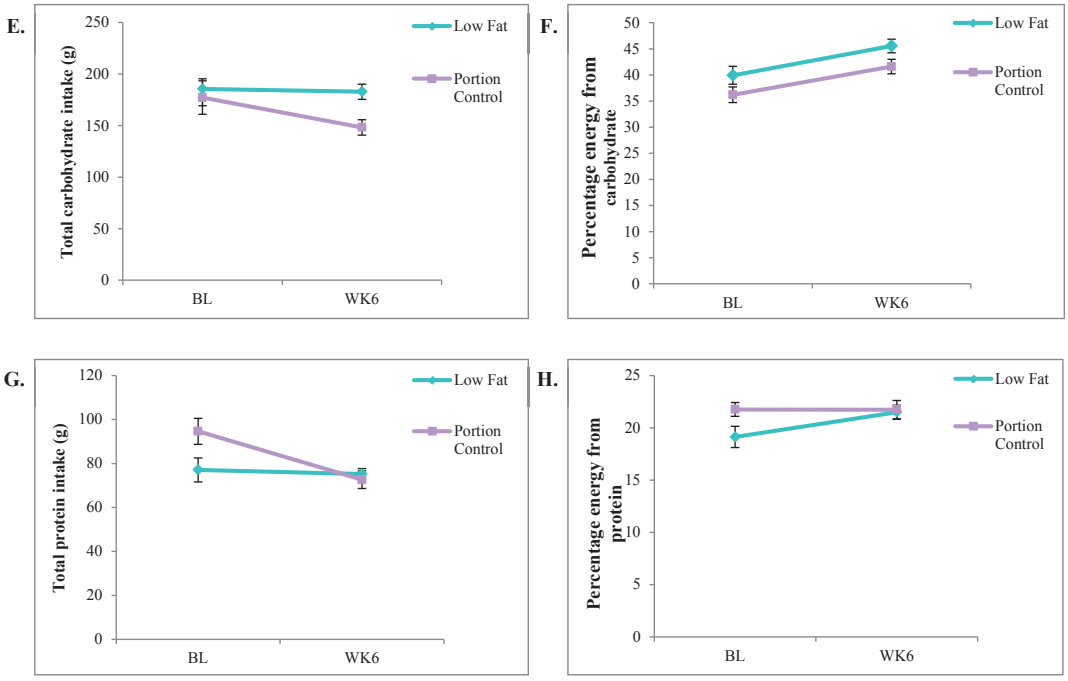


Figure 6.1 (cont.): Mean ± SEM BMI and macronutrient intake in the low-fat (n = 26) and portion control (n = 27) diet groups at baseline (BL) and week six (WK 6). **E.** total carbohydrate intake; **F.** percentage energy from carbohydrate; **G.** total protein intake; **H.** percentage energy from protein



6.5.4 Dietary questionnaires

Low-fat diet. During the intervention, the low-fat diet group significantly reduced their consumption of processed meat ($P = 0.044$), eggs ($P = 0.018$), dairy ($P = 0.038$), fried foods ($P = 0.008$), desserts ($P = 0.052$) and condiments ($P = 0.030$). Additionally, refined grains ($P = 0.038$) and leafy vegetables ($P < 0.001$) were consumed more frequently compared to at baseline.

Portion control diet. Subjects in the portion control diet group did not change their consumption of dairy, leafy vegetables, fried foods, desserts or condiments during the intervention, however there was a significant reduction in the frequency that processed meat ($P = 0.032$), eggs ($P = 0.021$) and chocolate ($P = 0.028$) were consumed.

The low-fat and portion control diet groups significantly differed in their consumption of eggs ($P = 0.050$), leafy vegetables ($P = 0.007$), other vegetables ($P = 0.011$) and fried foods ($P = 0.043$) during the intervention, with the low-fat diet group consuming eggs (LF: 0.08 times/ day, PC: 0.16 times/ day) and fried foods (LF: 0.08 times/ day, PC: 0.14 times/ day) on fewer occasions, and leafy vegetables (LF: 5.95 times/ day, PC: 4.48 times/ day) and other vegetables (LF: 3.87 times/ day, PC: 2.49 times/ day) on more occasions than the portion control group.

6.5.5 Physical activity

Physical activity levels did not differ at baseline between the low-fat diet group and the portion control group ($P = 0.227$). Subjects on either diet did not change their

physical activity levels during the study (main effect of time: Wilks' Lambda = 0.999, $F(1, 44) = 0.056$, $P = 0.815$). No significant differences were seen between groups (main effect of group: $F(1,47) = 2.26$, $P = 0.140$) and no significant time by group interaction was observed (time*group interaction: Wilks' Lambda = 0.984, $F(1, 44) = 0.700$, $P = 0.407$) (LF: BL, 11.41 ± 1.07 hr, WK6, 11.88 ± 1.19 hr; PC: BL, 14.77 ± 1.78 hr, PC, 13.95 ± 1.14 hr) from baseline to week six.

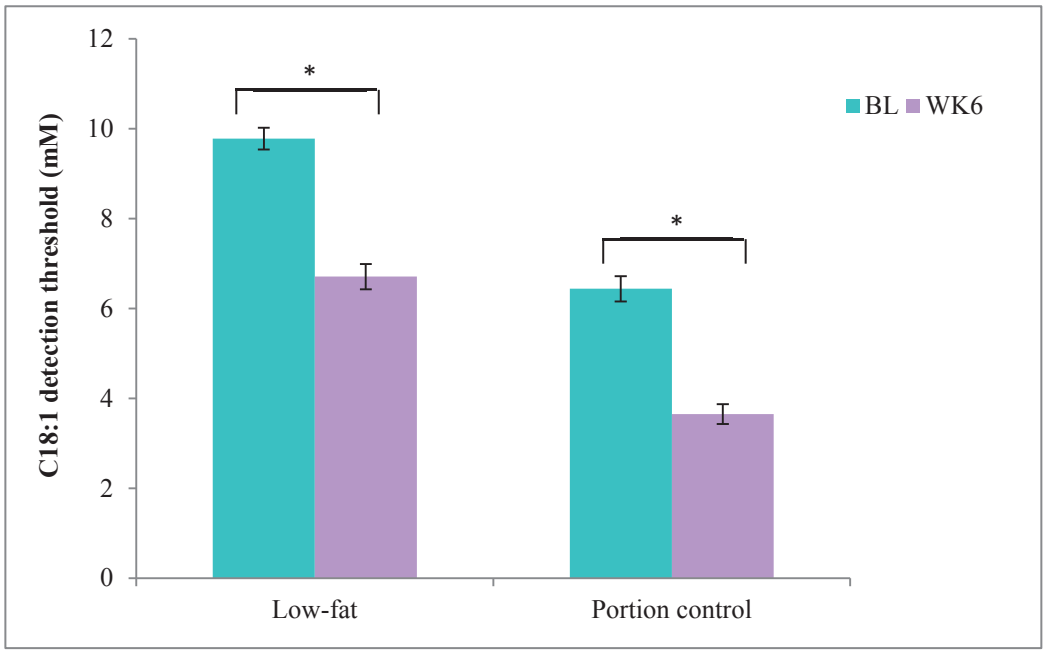
6.5.6 C18:1 detection thresholds

C18:1 taste thresholds did not differ between the two diet groups at baseline ($P = 0.654$) **(Table 6.2)**. Consumption of the low-fat and portion control diets over the six-week period significantly decreased C18:1 thresholds (LF: BL, 9.78 ± 0.24 mM, WK6, 6.71 ± 0.28 mM; PC: BL, 6.44 ± 0.28 mM, WK6, 3.65 ± 0.22 mM; main effect of time: Wilks' Lambda = 0.875, $F(1, 46) = 6.58$, $P = 0.014$), and there were no significant changes between groups (main effect of group: $F(1, 46) = 3.71$, $P = 0.060$) or interactions between time and group (time*group interaction: Wilks' Lambda = 0.999, $F(1, 46) = 0.039$, $P = 0.845$) **(Figure 6.2)**. Therefore, oral detection thresholds for C18:1 decreased in all subjects independent of the allocated diet.

There were no correlations between baseline oral fatty acid sensitivity and energy intake (BL: $r = 0.129$, $P = 0.363$; WK6: $r = 0.066$, $P = 0.636$), total fat intake (BL: $r = 0.014$, $P = 0.922$; WK6: $r = -0.080$, $P = 0.604$) or percentage energy from fat (BL: $r = -0.135$, $P = 0.340$; WK6: $r = -0.068$, $P = 0.652$) at baseline or week six. There were also no correlations between oral fatty acid sensitivity at week six and energy intake (BL: $r = 0.248$, $P = 0.089$; WK6: $r = 0.268$, $P = 0.065$), total fat intake (BL: r

= 0.085, P = 0.565; WK6: r = 0.060, P = 0.699) or percentage energy from fat (BL: r = -0.178, P = 0.226; WK6: r = -0.017, P = 0.913) at baseline or week six. A trend was found between baseline oral fatty acid sensitivity and baseline BMI (r = 0.252, P = 0.068) and a significant correlation was found between oral fatty acid sensitivity at week six and BMI at week six (r = 0.289, P = 0.046).

Figure 6.2: Mean ± SEM oral fatty acid detection thresholds at baseline (BL) and week six (WK6) for the low-fat (n = 26) and portion control (n = 27) diet groups



* Indicates significance $P < 0.05$ for main effect of time using repeated measures ANOVA

6.5.7 Sucrose and NaCl detection thresholds

There were no significant differences in baseline sucrose taste thresholds between the groups (P = 0.627). Similarly, there were no significant differences in baseline NaCl taste thresholds between the groups (P = 0.174) **(Table 6.2)**.

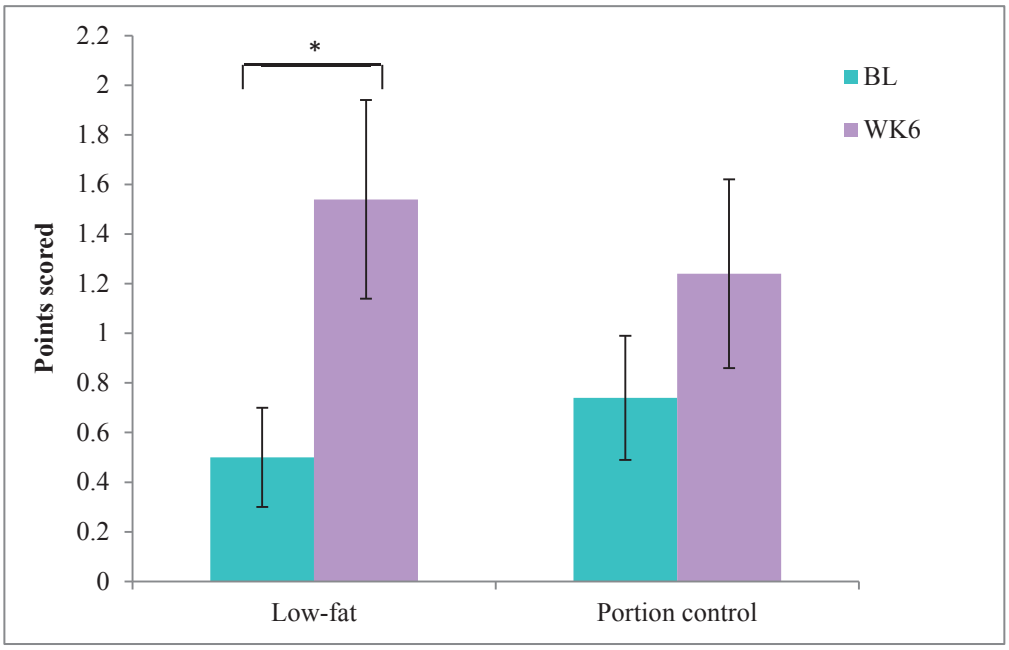
Consumption of the low-fat diet or the portion control diet over the six-week period had no significant effect on detection thresholds for sucrose (main effect of time: Wilks' Lambda = 0.969, $F(1, 47) = 1.50$, P = 0.227). No significant effect was seen for sucrose between groups (main effect of group: $F(1, 47) = 0.013$, P = 0.910) or time by group interaction (time*group interaction: Wilks' Lambda = 0.998, $F(1, 47) = 0.113$, P = 0.738) (LF: BL, 6.03 ± 0.93 mM; WK6, 5.12 ± 0.80 mM) (PC: BL, 6.50 ± 1.13 mM; WK6, 4.90 ± 1.12 mM). Therefore, detection thresholds for sucrose for all subjects did not change and this did not depend on what diet was followed.

There was no change in NaCl detection thresholds after consumption of the low-fat or portion control diet (main effect of time: Wilks' Lambda = 0.993, $F(1, 51) = 0.348$, P = 0.558). There were also no significant changes in NaCl detection thresholds between groups (main effect of group: $F(1, 51) = 1.63$, P = 0.208) or interactions between time and group (time*group interaction: Wilks' Lambda = 0.979, $F(1, 51) = 1.08$, P = 0.303) (LF: BL, 0.29 ± 0.04 mM; WK6, 0.07 ± 0.12 mM) (PC: BL, 0.41 ± 0.08 mM; WK6, 1.18 ± 0.93 mM), meaning that detection thresholds for NaCl remained the same for all subjects independent of diet type.

6.5.8 Fat ranking task

Scores on the fat ranking task at baseline did not differ between dietary groups (P = 0.348). There was a significant increase in the fat ranking task scores following the consumption of the low-fat diet (z = -2.38, P = 0.017) (BL: 0.50 ± 0.20, WK6: 1.54 ± 0.40) **(Figure 6.3)**. In contrast, consumption of the portion control diet had no effect on fat ranking task scores (z = -1.01, P = 0.314) (BL: 0.74 ± 0.25, WK6: 1.24 ± 0.38) **(Figure 6.3)**. A Mann-Whitney U test revealed no significant difference in the fat ranking task scores between the low-fat (Md = 0.5, n = 24) and portion control (Md = 0.0, n = 25) groups at week six (U = 264, z = -0.80, P = 0.42, r = 0.11). There were no significant differences between diets for the change in fat ranking scores from baseline to week six (U = 206, z = -0.62, P = 0.53, r = 0.09).

Figure 6.3: Mean ± SEM fat ranking scores at baseline (BL) and week six (WK6) for the low-fat (n = 26) and portion control (n = 27) diet groups



* Indicates significance $P < 0.05$ using Wilcoxon Signed-Rank tests

6.5.9 Hedonic ratings

The low-fat and portion control diet groups did not differ in liking of regular-fat and low-fat foods at baseline **(Table 6.4)**. Following the consumption of the low-fat diet, there were no differences in liking of regular-fat cream cheese, regular- and low-fat chocolate mousse, or regular- and low-fat yoghurt (main effect of time: Wilks' Lambda = 0.999, $F(1, 47) = 0.038$, $P = 0.847$; Wilks' Lambda = 0.990, $F(1, 47) = 0.465$, $P = 0.449$; Wilks' Lambda = 0.986, $F(1, 48) = 0.683$, $P = 0.413$; Wilks' Lambda = 0.271, $F(1, 47) = 0.271$, $P = 0.605$; Wilks' Lambda = 0.976, $F(1, 47) = 1.14$, $P = 0.291$, respectively), however there was a significant increase in the liking of low-fat cream cheese (main effect of time: Wilks' Lambda = 0.870, $F(1, 47) = 7.02$, $P = 0.011$). There was also no significant increase in liking of the foods between groups (main effect of group: $F(1, 47) = 1.35$, $P = 0.251$; $F(1, 47) = 0.969$, $P = 0.330$; $F(1, 47) = 0.957$, $P = 0.333$; $F(1, 47) = 0.060$, $P = 0.807$; $F(1, 47) = 3.71$, $P = 0.060$, regular- and low-fat cream cheese, low-fat chocolate mousse, or regular- and low-fat yoghurt, respectively) except for regular-fat chocolate mousse (main effect of group: $F(1, 47) = 4.31$, $P = 0.043$). No significant time by group interactions were observed for liking of the regular- and low-fat cream cheese, the regular- and low-fat chocolate mousse, or regular-fat yoghurt (time*group interaction: Wilks' Lambda = 0.960, $F(1, 47) = 1.98$, $P = 0.166$; Wilks' Lambda = 0.981, $F(1, 47) = 0.927$, $P = 0.341$; Wilks' Lambda = 0.969, $F(1, 47) = 1.50$, $P = 0.227$; Wilks' Lambda = 0.997, $F(1, 47) = 0.126$, $P = 0.725$; Wilks' Lambda = 0.997, $F(1, 47) = 0.149$, $P = 0.702$, respectively), however there was a time by group interaction for liking of the low-fat yoghurt (time*group interaction: Wilks' Lambda = 0.913, $F(1, 47) = 4.48$, $P = 0.040$). This significant interaction may be due to the

portion control diet group rating the low-fat yoghurt higher than the low-fat diet group at baseline.

Table 6.4: Acceptance changes (Mean ± SEM) using a 9-point hedonic scale in regular- and low-fat foods following consumption of the low-fat diet or portion control diet

		LF (*n* = 26)	PC (*n* = 27)	*P* value
RF chocolate mousse				
	Baseline	7.2 ± 0.3	6.3 ± 0.4	0.083[a]
	Week 6	7.3 ± 0.3	6.2 ± 0.3	0.449[b]
	Change	0.1 ± 0.3	-0.1 ± 0.3	
LF chocolate mousse				
	Baseline	6.0 ± 0.3	5.6 ± 0.4	0.418[a]
	Week 6	6.2 ± 0.4	5.9 ± 0.4	0.413[b]
	Change	0.2 ± 0.5	0.3 ± 0.5	
RF yoghurt				
	Baseline	6.4 ± 0.3	6.2 ± 0.4	0.769[a]
	Week 6	6.4 ± 0.4	6.6 ± 0.3	0.605[b]
	Change	0.0 ± 0.4	0.4 ± 0.5	
LF yoghurt				
	Baseline	5.6 ± 0.4	6.8 ± 0.3	0.011[a]
	Week 6	5.7 ± 0.4	6.0 ± 0.3	0.291[b]
	Change	0.1 ± 0.4	-0.8 ± 0.3	
RF cream cheese				
	Baseline	5.6 ± 0.4	6.5 ± 0.4	0.156[a]
	Week 6	5.8 ± 0.5	6.0 ± 0.4	0.847[b]
	Change	0.2 ± 0.4	-0.5 ± 0.3	
LF cream cheese				
	Baseline	5.5 ± 0.4	5.6 ± 0.4	0.923[a]
	Week 6	6.0 ± 0.3	6.8 ± 0.3	0.011[b]
	Change	0.5 ± 0.5	1.2 ± 0.4	

RF, regular-fat; LF, low-fat

[a] indicates *P* values for the difference between groups in baseline scores using independent samples t-tests

[b] indicates *P* values for a main effect of time using repeated measures ANOVA

6.5.10 PROP sensitivity

There was no significant difference in PROP sensitivity between the groups at baseline (P = 0.242). There was no significant difference in changes in PROP sensitivity between the low-fat and portion control diet groups from baseline to week six (main effect of time: Wilks' Lambda = 1.00, F(1, 47) = 0.001, P = 0.972). There were also no significant changes between groups (main effect of group: F(1, 47) = 0.121, P = 0.730) or group by time interactions (time*group interaction: Wilks' Lambda = 0.973, F(1, 47) = 1.32, P = 0.256) (LF: BL, 16.59 ± 3.06, WK6, 18.02 ± 2.96; PC: BL, 19.46 ± 3.15, WK6, 18.12 ± 3.07).

There were no significant correlations between PROP sensitivity and total fat intake (g) at baseline (r = 0.035, P = 0.807) or week six (r = 0.029, P = 0.853), or percentage energy from fat at baseline (r = -0.146, P = 0.316) or week six (r = -0.039, P = 0.793), nor were there any correlations between PROP sensitivity and oral fatty acid sensitivity (r = -0.048, P = 0.744), or PROP sensitivity and ability to detect the fat content of custard (r = 0.184, P = 0.206).

6.5.11 Tongue papillae number

There was no significant difference in papillae number between the low-fat diet group and the portion control diet group at baseline (LF: 1.82 ± 0.41, PC: 2.65 ± 0.54, P = 0.259). There was no correlation between fungiform papillae number and oral fatty acid sensitivity (BL: r = -0.119, P = 0.447, WK6: r = -0.002, P = 0.990). No significant correlation was found between fungiform papillae number and detection thresholds for sucrose (BL: r = -0.173, P = 0.267, WK6: r = -0.149, P =

0.339) or NaCl (BL: r = -0.031, P = 0.846, WK6: r = 0.256, P = 0.097). There was no correlation between fungiform papillae and ability to perceive fat in the fat ranking task (BL: r = 0.013, P = 0.934, WK6: r = 0.141, P = 0.369). There was also no correlation found between fungiform papillae number and sensitivity to PROP (BL: r = 0.249, P = 0.108, WK6: r = 0.210, P = 0.175).

6.6 Discussion

This study compared the effects of following a six-week low-fat diet versus a six-week portion control diet on oral detection thresholds for C18:1, sucrose and NaCl; and fat perception and hedonic ratings for regular-fat and low-fat foods. Oral fatty acid sensitivity was significantly increased after consumption of both the low-fat diet and portion control diets and this increase in sensitivity was specific to C18:1; sensitivity to sucrose and NaCl did not change. Fat perception significantly increased following a low-fat diet, but the same changes were not seen following the portion control diet. There were minimal significant changes in hedonic ratings from baseline to week six for either dietary intervention, and preferences for regular-fat and low-fat foods were not significantly different between groups at week six.

Oral fatty acid sensitivity increased significantly in both the low-fat and portion control diet groups from baseline to week six, and no significant differences between diet groups were found, suggesting that an adaptive change to both diets occurred during the six-week period. At baseline, all subjects were overweight or obese and were habitually consuming moderate fat diets (33-34% fat). Both the low-fat diet group and the portion control diet group reduced the gram value of fat consumed

(LF: -22.7 g, PC: -16.8 g). The present study demonstrates that subjects who reduce their fat intake by approximately 20 g via consumption of a moderately low-fat diet or a portion control diet for six weeks will see similar changes in sensitivity with an improvement in detection thresholds of approximately 2.9 mM concentration of C18:1. The significant changes to both groups were expected, as the portion control group were instructed to reduce the amount of all foods they were consuming; consequently, the amount of fat (g) was reduced. It could be speculated that these changes were due to a learning effect of the detection threshold method rather than modulation by fat intake, however a learning effect is unlikely as no differences in oral sensitivity to sucrose and NaCl were observed from baseline to week six, and we have previously reported the high test-retest reliability of oral fatty acid detection thresholds (Newman & Keast, 2013). Previous research has reported similar findings in that reducing the amount of dietary fat consumed (grams and percentage) improves oral sensitivity to fat (Stewart & Keast, 2012). Stewart & Keast (2012) detailed that a 56 g reduction in fat consumption resulted in a 2.2 mM increase in oral sensitivity to C18:1 over a four-week period, suggesting that the gram value of fat consumed in the diet may be an important factor in modulating oral fatty acid sensitivity. Although the low-fat diet group tended to consume a lower percentage of energy from fat (24.9%), compared to the portion control group (29.2%) ($P = 0.058$) at week six of the present study, there were no significant differences found between diets. Therefore, even though the low-fat diet group were consuming a lower percentage of energy from fat, they were consuming approximately the same amount of grams in fat. This reflects the fact that no differences were found in oral fatty acid sensitivity between the diets and further suggests that the gram value of fat is the

major influence on oral fatty acid sensitivity, rather than the percentage energy from fat. Thus, it could be hypothesised that if these reduced-fat diets are followed habitually, individuals may become more sensitive to fats over time and may require smaller amounts of fat/ fatty acids to elicit the same response within taste receptor cells. If taste receptor cell activation is linked with development of satiety, then increasing oral sensitivity to fatty acids may aid in reducing total energy intake and possibly obesity. However, it is important not to discount the potential effect of weight loss. As modulation of oral fatty acid sensitivity is yet to be shown independent of weight loss or weight gain, this will be an important area of future investigation with studies needed which can alter fat intake without inducing weight loss, for example a moderate-fat, high kilojoule diet. Future research may include a follow-up study to determine the long-term effects of such an intervention where subjects are re-tested after six months or one year, asked about their continued adherence to their originally allocated diet and again undergo detection threshold testing. Additionally, comparing a low-fat diet to a control diet in which fat intake is not reduced, *e.g.,* a Mediterranean diet, may allow for clearer comparisons in oral fatty acid sensitivity between groups.

The present study found no direct correlation between oral fatty acid sensitivity and fat intake (either in grams or percentage). This finding is surprising as oral fatty acid sensitivity appeared to increase following a decrease in dietary fat consumption. Previous data surrounding this area is conflicting, with some studies finding strong associations between oral fatty acid sensitivity and habitual fat intake (Stewart, et al., 2010; Stewart, Newman, et al., 2011), and others finding no associations (Tucker, et

al., 2013; Tucker & Mattes, 2013). Stewart et al. reported in two separate cross-sectional studies that hyposensitivity to fatty acids was associated with higher habitual fat consumption (Stewart, et al., 2010; Stewart, Newman, et al., 2011) and another research group failed to find cross-sectional associations between oral fatty acid sensitivity and dietary fat consumption over ten testing sessions (Running, et al., 2013). Additionally, a comparison of fat intake and oral fatty acid sensitivity in lean and obese subjects found no differences or associations (Stewart & Keast, 2012). These findings are surprising, given overweight/ obese individuals are thought to have attenuated sensitivity due to diets habitually high in fat. However, the type of data presented in these studies may provide insight into how oral fatty acid sensitivity may be modulated by fat intake. Cross-sectional data may not be the most effective way to determine the influence of diet on oral fatty acid sensitivity. For instance, if two subjects who both consume excess dietary fat undergo a single session of oral fatty acid sensitivity testing, one subject's threshold may be 12 mM and the other 2 mM due to expected inter-individual variability; this cross-sectional data would show no correlation between oral fatty acid sensitivity and fat intake. However, if the same subjects both followed a low-fat diet, their thresholds may change to 6.4 mM and 1 mM respectively, demonstrating an association between fat intake and oral fatty acid sensitivity. Consequently, the results of the present longitudinal study suggest that there is empirical evidence that fat intake and oral fatty acid sensitivity are linked, although the area surrounding habitual diet and its association with oral fatty acid sensitivity is conflicting at this stage. Future studies involving large, long-term interventions that monitor habitual fat consumption, as

opposed to cross-sectional studies, are needed to determine a definitive answer as to whether fat intake modulates oral fatty acid sensitivity or *vice versa*.

The present study found a correlation between BMI and oral sensitivity to C18:1 at week six. Previous research has demonstrated associations between oral fatty acid sensitivity and BMI (Stewart, et al., 2010; Stewart, Newman, et al., 2011), however other studies have found no associations (Mattes, 2009d, 2011b; Stewart & Keast, 2012). The studies that have found associations between the two have used an abbreviated threshold testing method that divided subjects into hypersensitive and hyposensitive based on their sensitivity to a certain concentration of C18:1 (Stewart, et al., 2010; Stewart, Newman, et al., 2011; Stewart, Seimon, et al., 2011). In contrast, the studies that have found no associations have used a complete threshold measure procedure (3-AFC, staircase methodology *etc.*) (Kamphuis, Saris, et al., 2003; Mattes, 2009d, 2011b; Stewart & Keast, 2012). To the best of the author's knowledge, the present study is one of the first studies to find an association between BMI and oral fatty acid sensitivity using a complete threshold testing method (triangle tests with ascending forced choice methodology). This suggests that perhaps there is a link between the two and attenuation of the fat sensing mechanism in the oral cavity is compromised in the obese population.

Interestingly, only C18:1 detection thresholds changed over the six-week period for both groups. There were no changes in oral detection thresholds for sucrose or NaCl, nor were there changes in sugar and sodium intake over the six week period. This adds to the body of evidence that taste perception is influenced by specific nutrient

intakes and therefore, is suggestive that fat perception is potentially modulated specifically by fat intake. These tests were used to control for potential learning effects that may have occurred from session one to session two, and also to determine if other taste related changes occurred. As there were no changes in oral detection thresholds for sucrose and NaCl, it can be suggested that the increases in oral fatty acid sensitivity were specific to the decreased intake of fat throughout the six-week period.

Whilst significant reductions in oral detection thresholds were seen in the both the low-fat and portion control diet groups during the intervention, only the low-fat diet group demonstrated a significant increase in ability to detect the fat content of custard from baseline to week six. Increases in fat perception in the portion control group were also seen, however these increases were not significant. This suggests that directionality in fat perception was similar between the two diet groups but the magnitude of change was not large enough to see a statistical difference. A larger sample size or a longer intervention period may have produced statistically significant differences in the portion control group. Differences between groups in regards to changes in fat perception may have also been due to the types of foods that were being consumed throughout the intervention. Subjects following the portion control diet were instructed to consume the same types of foods as normal but at reduced quantities. Therefore, the portion control group consumed high-fat foods such as cheese, fried foods, desserts and condiments significantly more often during the intervention than the low-fat group. Consumption of these types of foods throughout the six week period may account for the fact that there was no significant

increase in fat perception in the portion control group. This finding highlights the possibility that the types of food habitually consumed may positively affect oral perception in an overweight/ obese individual reversing the attenuation of fatty acid sensitivity that may have occurred in the alimentary canal. Previous research has also found similar results, with an increase in fat perception observed after consumption of a low-fat diet (15-20%) over four weeks (Stewart & Keast, 2012). However, in the current study there were no significant differences between groups at week six in their ability to perceive fat in the custard samples.

Minimal changes in hedonics for the foods tested were found in this study. The sole statistically significant change was increased liking of low-fat cream cheese in both groups across the intervention, however both groups demonstrated slight non-significant increases or decreases in preference of some foods pre- and post-intervention. The data show that all foods were liked in a similar manner both at baseline and week six. In a study conducted by Stewart et al. (2012) with a duration of four weeks, there were also no changes in liking of regular- and low-fat foods. A longer 10-12 week low-fat dietary intervention may be needed to replicate the increases in liking of low-fat foods as previously reported by Mattes (1993) and in order for these adaptive changes to be sustainable in the long-term, a change in preference (increased liking of low fat foods) would need to be seen (Mattes, 1993). Whilst speculative at this stage, hyposensitivity to fatty acids may be due to habituation to a high-fat diet which weakens the chemoreceptive response in the oral cavity. Therefore, hypothetically, if changes are made to reduce the amount of fat being consumed over a long period of time, regular chemoreceptive responses may

be restored, potentially reducing over consumption and overall body weight as shown in this study and previous work by Stewart and Keast (2012). Whilst unconfirmed in the literature at this stage, habituation of the taste system in some individuals may have occurred in response to the changing food environment, *i.e.,* increased availability of high-fat foods, which has potentially resulted in differences in sensitivities to fats, thus creating the need for greater quantities of fat to be consumed before detection occurs, consequently resulting in excess consumption. The results of the present study do not clearly support this position, however being able to make the links between fat perception, fat preference and oral fatty acid sensitivity is an important step in establishing the potential fundamental role of nutrient-specific receptors both in the mouth and GI tract, and how these receptors influence eating behaviours and perhaps overconsumption, or *vice versa*. Hence, there is a possibility that differences in oral fatty acid sensitivity may also affect food choices and preferences. Nonetheless, for a change in habitual diet to be sustained long-term, a change in preference needs to occur.

While this study focuses on dietary influences, it is important to note that the ability to detect fatty acids may differ between individuals and this may be due to differences in fatty acid receptor functionality or papillae density (Chale-Rush, et al., 2007b; Gilbertson, et al., 2005). For example, those who have a higher density of papillae will have a greater number of taste receptors and potentially increased oral sensitivity to many nutrients (Drayna, 2005; Hayes, et al., 2010). Furthermore, differences in sensitivity to certain tastants may influence liking or dislike of certain foods containing those tastants (Drewnowski & Henderson, 2000; Keller, et al.,

2002; Turnbull & Matisoo-Smith, 2002). As fatty acid receptor numbers may differ from person to person, oral fatty acid sensitivity may also differ between humans (Kamphuis & Westerterp-Plantenga, 2003), potentially affecting the hedonic response to fats. However, receptors for fatty acids have not yet been isolated on fungiform papillae. The present study did not find any relationship between fungiform papillae number and sensitivity to C18:1, sucrose, NaCl or PROP. The absence of such associations may be due to the participants' papillae numbers, which were much lower than previously reported (Nachtsheim & Schlich, 2013; Shahbake, et al., 2005). Differences in methodology may account for the low papillae numbers as laboratories use varying methodologies to determine papillae numbers. The photographic technique used in the present study has been used by many laboratories however, there are different ways it can be carried out. Some groups dye half of the tongue, then take a photograph (Nachtsheim & Schlich, 2013). The area of the tongue which is to be counted is then determined once the image is on a computer. Therefore the researchers could potentially use the area which looks as though it contains the most papillae. However, others research groups place specific sized circles of filter paper on the tongue at the time of the photograph and only count the papillae in that particular area (Shahbake, et al., 2005). Thus, if the circle marker is placed in an area of the tongue which is less populated with papillae, counts may be lower, as was the case for the present study, than the computer based studies. Additionally, the use over cover slips is common and this helps to identify non-stained papillae, however these were not used in the present study.

This study needs to be considered alongside limitations which may have confounded the results. Dietary adherence is very difficult to monitor during interventions that continue over many weeks, however the significant reduction in weight, BMI and waist-hip ratio, along with responses to the food frequency questionnaire suggest that subjects adhered to their allocated diet. Diet records were used to provide a snapshot of dietary intake both before and during the intervention, however they do not necessarily reflect habitual dietary behaviour (Potosky, Block, & Hartman, 1990). Diet records often underreport socially undesirable foods, which also tend to be high in fat (Voss, Kroke, Klipstein-Grobusch, & Boeing, 1998), however as subjects would have underreported at both baseline and week six, it is thought the changes in fat intake (g) and fat percentage intake in the present study are real changes. Discrimination of a true oral detection effect from fatty acids may be confounded by differences in the textural attributes of fatty acid and control samples, which can influence oral detection. However, we used reliable methods to minimise textural differences, odour differences and oxidation, therefore any influence would be expected to have been minor (Mattes, 2005). It must also be mentioned that both the low-fat diet group and the portion control diet group reduced the amount of fat they were consuming from baseline to week six. Therefore, comparison between the two groups in regards to changes in oral fatty acid sensitivity are difficult as there were no significant differences in fat intake, both in grams and percentage, at week six between the two groups.

6.7 Conclusions

In summary, this study has demonstrated that following either a six-week low-fat diet or portion control diet significantly increases oral sensitivity to C18:1. These findings suggest that reducing the total grams of fat consumed has a positive effect on an individual's oral fatty acid sensitivity. Individuals who do not make these dietary changes may be subject to gustatory adaptive changes that may reduce the physiological and psychological effects of fat, and potentially contribute to excess fat consumption. Therefore, habitual fat consumption may be a major factor in influencing an individual's sensitivity to fat, and in turn either promote or reduce the likelihood of the development of overweight and obesity. However, the present study also raises the possibility that a change in fat intake rather than the absolute content of fat consumed may be an important factor in determining oral fatty acid sensitivity. In addition, whether there is an underlying influence from genetics on taste sensitivity is yet to be elucidated, thus, future studies are needed to ascertain the potential role of variations in receptors and expression of fatty acid specific receptors.

6.8 Future directions

To extend this research, the next step would be to investigate the changes in habitual food consumption and preference following dietary adaptation over an extended period of time to investigate the potential changes in preference. In addition, a follow-up study whereby the same subjects are re-tested six months to a year later to ascertain which subjects have continued to adhere to the diet, and whether this extended adherence has influenced oral fatty acid sensitivity would be interesting.

Another weight loss dietary intervention where the control diet group follow a diet that does not reduce fat intake, for example a Mediterranean diet, would allow a clearer comparison between groups in regards to oral fatty acid sensitivity, and associations between sensitivity and fat intake may be more obvious. Future studies investigating modulation of oral fatty acid sensitivity through dietary changes will need to control for energy intake as differences in sensitivity need to be reported without changes in energy intake occurring, so a definitive link between fat intake and sensitivity can be made. Another important step would be to investigate the effect of long-term dietary adaptation on satiety to both low- and high-fat foods. Importantly, future research must focus on the putative receptors for fatty acid sensitivity, how expression of these receptors varies from person to person, and what happens when dietary consumption is altered.

CHAPTER SEVEN

Summary of major findings and conclusions

7.1 Introduction

Excess fat consumption is the leading cause of obesity and thus contributes to various non-communicable diseases such as cardiovascular disease (CVD) and type-2 diabetes (World Health Organisation, 2000). Although the area of oral fatty acid detection is novel, there is emerging evidence that humans may have a mechanism for sensing fat in the oral cavity which occurs via fatty acid receptors on taste buds. Whilst there is much speculation over what constitutes a primary taste quality, a proposed criteria has been established comprising of: defined class of stimuli; unique transduction mechanism; peripheral taste mechanism; and be distinguishable from other taste stimuli (Mattes, 2011a). Fatty acids seem to fit all of these criteria, however challenges arise with distinguishing fatty acid stimuli from other taste stimuli as there is no established lexicon for fatty acid detection, making testing difficult for subjects who are experiencing the stimuli for the first time (Running, et al., 2013). Similarly to the primary tastes, there is large inter-individual variation in the ability to detect fatty acids within the oral cavity, whereby some individuals have the ability to detect fatty acids at low concentrations, while others need higher concentrations before fatty acid detection occurs (Ahne, et al., 2000; Cicerale, et al., 2009; Mueller, et al., 2011; Newman & Keast, 2013). An individual's preference for high-fat food may be influenced by their oral sensitivity to fatty acids, however whether preference determines oral sensitivity or *vice versa* is still unknown. Those who are hypersensitive to fatty acids may consume less fat and total energy, whereas

those who are hyposensitive may consume more fat and total energy (Stewart, Newman, et al., 2011). We suggest this association may be due to gastrointestinal (GI) sensitivity to fatty acids, in which potent stimulators of the satiety hormone cascade are affected (Stewart, Seimon, et al., 2011). As fat consumption contributes to weight gain and obesity is largely becoming an epidemic, factors that may influence the consumption and preference of fatty foods – including fatty acid sensitivity – are important considerations. As there are many gaps in the current knowledge of fatty acid sensitivity, this thesis endeavoured to expand on, and add to the growing body of evidence of this novel research area.

Variation in oral sensitivity to fatty acids has been consistently reported in both animal and human studies, with inter-individual variation becoming a well-documented characteristic of the taste system (Newman & Keast, 2013; Running, et al., 2013; Tucker & Mattes, 2013). The mechanisms behind variation in individual sensitivity to fatty acids remains elusive, but is thought to be due to a combination of genetic factors (Gilbertson, et al., 2005), including the number of taste papillae, which may be related to a heightened taste perception, and environmental factors, for example, diet-induced changes in taste receptor functionality via adaptation. Previous studies have reported changes in the oro-sensory perception of tastants including 6-n-propylthiouracil (PROP), salt and fats, as well as the liking of foods containing certain tastants following dietary manipulations. For example, individuals classed as supertasters of the bitter compound PROP may avoid certain bitter tasting foods such as brassica vegetables, bitter tasting fruits (grapefruit) and coffee (Dinehart et al., 2006; Drewnowski & Henderson, 2000; Drewnowski, Henderson,

Levine, & Hann, 1999; Drewnowski, Henderson, & Shore, 1997; Hall, Bartoshuk, Cain, & Stevens, 1975). Prior research has also found a relationship between sodium consumption and taste sensitivity to sodium, whereby high dietary sodium consumption is associated with a lower taste sensitivity to sodium (DiNicolantonio, et al., 1984). It is possible that a similar relationship may exist with fat. Stewart et al. (2011) demonstrated that fat hypersensitivity was associated with a lower dietary fat consumption, which suggests a potential link between high dietary fat consumption and decreased oral fatty acid sensitivity (Stewart, Newman, et al., 2011). Mattes et al. (1993) evaluated the relationship between habitual fat consumption and the preferred level of fat in foods, finding that after consumption of a fat-reduced diet, preference for previously preferred full-fat foods decreased and acceptance of lower-fat foods increased (Mattes, 1993). Thus, in some circumstances, the environment may override an individual's inherent oral sensitivity via adaptation, suggesting a direct relationship between habitual fat consumption and the level of fat preferred in a food, and perhaps oral sensitivity to fatty acids. Further exemplifying these interactions, decreases in oral fatty acid sensitivity were seen after consumption of a four-week high-fat diet, and oral fatty acid sensitivity increased following a four-week low-fat diet (Stewart & Keast, 2012). If an individual is repeatedly exposed to a high-fat diet, attenuation of the physiological and psychological effects of fat may occur. Accordingly, it may be plausible that prolonged consumption of a high-fat diet will lead to a decrease in oral sensitivity to fatty acids, and consumption of more fat will be required to obtain the expected release of appetite supressing hormones and increased satiety.

Recently, a homologous taste receptor response for sugars and proteins was discovered throughout the GI tract, illustrating a systemic nutrient detection system for carbohydrates and proteins (Margolskee et al., 2007). Similarities between fatty acid receptors on papillae and GI tract epithelial cells have been found, including cluster of differentiation 36 (CD36), G-protein coupled receptors (GPCR) and delayed rectifying potassium (DRK) channels, suggesting fat is also part of the systemic nutrient detection system throughout the alimentary canal. The role of gut nutrient sensing plays a pivotal role in appetite regulation and has been linked to digestive, metabolic and satiating effects involved in nutrient utilisation and inhibition of appetite (Sclafani & Ackroff, 2012). Indeed, when healthy individuals ingest fat, various hormones are released in preparation for fat digestion including cholecystokinin (CCK) and peptide YY (PYY), which potently affect appetite suppression by decreasing GI transit time (Feinle et al., 2003; Stoeckel, Weller, Giddings, & Cox, 2008). Conversely, in the obese population, the GI regulation of food may be dysfunctional with accelerated, normal and delayed gastric emptying all being reported, as well as lower concentrations of PYY (Jackson et al., 2004; le Roux et al., 2006; Vazquez Roque et al., 2006; Verdich et al., 2000). The explanation for abnormalities in these mechanisms remains elusive, however it is hypothesised that desensitisation of the GI tract receptors may have occurred, possibly due to adaptation to dietary conditions, for example a high-fat diet (Little, Horowitz, & Feinle-Bisset, 2007). Stewart et al. (2011) investigated the link between fatty acid sensitivity in both the oral cavity and GI tract in lean and obese individuals, reporting that the obese were less orally sensitive to C18:1, had a reduced release of appetite supressing hormones after an intra-duodenal infusion of

C18:1 compared to the lean group and consumed more energy in the meal following the fat infusion (Stewart, Seimon, et al., 2011). (Dinehart, et al., 2006; Mattes, 2009b; Sternini, Anselmi, & Rozengurt, 2008). Similarities in mechanisms involved in sweet, bitter, umami and fat detection between taste receptor cells and GI cells have been reported whereby associations between oral sensitivity to certain tastants, dietary intake and preferences were found (Dinehart, et al., 2006; Mattes, 2009b; Sternini, et al., 2008). This suggests that the oral cavity and GI tract interact very closely to control appetite regulation and energy intake. However, further studies are required to determine the relationship between GI and oral fatty acid receptor functionality and density, and whether they are potentially influenced by dietary fat consumption.

Fat is a slow satiating but high density food compared to other macronutrients such as carbohydrates and proteins (Westerterp-Plantenga, 2003). For example, the mechanisms that induce satiation by fats are delayed in comparison with those induced by carbohydrates and proteins (Blundell & Macdiarmid, 1997). When ingested, fats have a potent effect on many peptide hormones and when digested changes in gastric motility occur which lead to appetite suppression (Feinle, et al., 2003; Stoeckel, et al., 2008). It has been found that these pathways are defective in the obese population (Stewart, Seimon, et al., 2011), meaning they are less satiated by fatty foods due to their decreased sensitivity to fats within the GI tract. Due to this inability to produce satiation quickly, excess fat may be consumed, increasing total energy consumption and consequently causing obesity (Snoek, et al., 2004). In addition, high-fat diets are usually more appealing than low-fat diets due to the high

palatability of fat (Drewnowski, et al., 1992). Drewnowski et al. (1992) showed that hedonic ratings for fat increased with body mass and that obese subjects had a stronger preference for high-fat foods when compared to leaner subjects (Drewnowski, et al., 1992). Similar results were found in a study comparing weight-discordant monozygotic twins, whereby the obese twins reported their current preference for fatty foods as three times higher than the co-twin (Rissanen, et al., 2002). These data suggest there is an environmental component for fat preference, and possibly a link between increasing body mass and an increased preference for high-fat foods. However, not all studies have reported the same results, with one study finding no differences in preference for regular-fat and low-fat foods between lean and obese subjects (Stewart & Keast, 2012). Post-ingestive effects of fat including feelings of contentment may promote long-term preference and positive reinforcement (Abumrad, 2005). Therefore, fat consumption produces feelings of pleasure and satisfaction, which then become positively associated with the fatty foods, and over time these foods become highly preferable (Abumrad, 2005). The obese population's preference for high-fat foods may be due to a decline in oral sensitivity to fat via adaptation, which may mean more fat must be consumed to produce the same hedonic response, potentially increasing fat consumption and increasing BMI. However, a robust relationship between BMI and oral fatty acid sensitivity is yet to be demonstrated, with recent research finding no relationship between the two (Kamphuis, Saris, et al., 2003; Mattes, 2009d, 2011b; Newman & Keast, 2013; Stewart & Keast, 2012).

An essential step in furthering evidence for fatty acid taste was establishing the reliability and reproducibility of the oral fatty acid threshold method. Whilst there is large speculation over the reliability of the fatty acid detection measurement, we have recently established that the measure is highly reproducible over a number of testing sessions (Newman & Keast, 2013). The study found that the most reliable and convenient measurement to ascertain the most accurate portrayal of a subject's sensitivity to C18:1 was to perform the threshold test in duplicate on the same day. However, other studies have found that numerous sessions (up to seven) over numerous days (up to ten) are required to determine an accurate measure of oral fatty acid thresholds (Tucker & Mattes, 2013). Tucker and Mattes (2013) investigated the reliability of oral fatty acid thresholds using two regularly used methodologies; the staircase method and the ascending 3-alternate forced choice (3-AFC) method. It was reported that subjects in this study improved their threshold measure with the more sessions they completed, thus the authors suggest that up to seven sessions may be required before an individual's threshold remained stable (Tucker & Mattes, 2013). These results are markedly different to those found within this thesis, which may be due to differences in methodologies used as well as differences in the vehicle used to present the fatty acids. However, this study raises some important suggestions surrounding the usefulness of specific methods, suggesting that the ascending 3-AFC method may be best used when categorising subjects into hypersensitive and hyposensitive, whereas the staircase method may be best used to determine stability in threshold measures (Tucker & Mattes, 2013). With that in mind, future research may need to further investigate the stability and determine whether the differences in findings between studies do lie with methodological and vehicle differences. There

are also questions surrounding the effect that the meal consumed preceding the oral fatty acid sensitivity test may have on the threshold measure. Some studies had previously found that taste sensitivity to sucrose, sodium chloride (NaCl) and quinine increased after a meal, while others had reported they had decreased and others reported no change (Pangborn, 1959; Pasquet, et al., 2006; Zverev, 2004). To the best of the author's knowledge, no studies have investigated the potential effect of the macronutrient composition of the meal consumed immediately prior to threshold testing. The results from this thesis have found that there was no significant effect of a high-fat, low-fat or macronutrient balanced breakfast on oral detection thresholds for C18:1. The major findings from this thesis are as follows:

Study one hypothesised that oral fatty acid thresholds for C18:1, linoleic acid (C18:2) and lauric acid (C12:0) would be as reliable and reproducible as thresholds for sucrose, citric acid, NaCl, caffeine and monosodium glutamate (MSG). This study reported that detection thresholds for saturated and unsaturated fatty acids could be reliably established for all participants within a micro- to millimolar concentration range in a group of healthy individuals over numerous testing sessions. The test-retest reliability of fatty acid detection thresholds mimicked the reliability of the five primary tastes which has been tested both in this study and in previous research (Ahne, et al., 2000; Cicerale, et al., 2009; Mueller, et al., 2011). Oral sensitivity to C18:1, C18:2 and C12:0 was not associated with oral sensitivity to the five primary taste qualities; sucrose, citric acid, MSG, NaCl or caffeine. This finding was expected as there are structural differences between taste molecules, and individuals may be more or less sensitive to structurally different tastants due to

potential differences in receptor systems (Keast, Bournazel, & Breslin, 2003). The findings of Study one also support previous research in that saturated and unsaturated fatty acids can be detected within the oral cavity of humans at low concentrations when non-taste cues, for example, odour, texture and visual prompts are minimised. In addition, we report that the most reliable and convenient way to accurately determine an individual's sensitivity to fatty acids was to measure detection thresholds in duplicate on the same day. It is important to note that measuring detection thresholds across days or in triplicate was also highly reliable.

Study two hypothesised that the consumption of a high-fat, low-fat or macronutrient balanced breakfast immediately prior to threshold testing would not have an influence on oral sensitivity to C18:1. This study established that the macronutrient composition of the meal consumed one hour prior to detection threshold testing did not influence oral fatty acid detection thresholds. Detection thresholds for C18:1 were not significantly different after the consumption of a high-fat, low-fat or macronutrient balanced breakfast one hour preceding detection threshold testing, suggesting that a one off high-fat meal does not play a functional role in determining an individual's oral sensitivity to fatty acids. In addition, we report that no changes in fat perception or preference for regular-fat or low-fat foods were seen after the three different meals. These findings again suggest that short-term dietary fat consumption does not influence oral fatty acid sensitivity, fat perception and preferences for regular-fat and low-fat foods.

Study three hypothesised that the gustatory system would show a degree of modulation in relation to oral sensitivity to C18:1 in a similar way to that of NaCl, in that reducing consumption of NaCl increases sensitivity to NaCl. This study reported that exposure to either a low-fat diet or a portion control diet for six weeks could modulate oral fatty acid sensitivity in overweight and obese individuals. In addition, it has been found previously in healthy weight subjects that consumption of an extreme high-fat diet (> 45% total energy from fat) for four weeks decreases oral sensitivity to fatty acids, and consumption of an extreme low-fat diet (< 20% total energy from fat) for four weeks increases oral fatty acid sensitivity. Therefore, we report that after consumption of a low-fat (25% total energy from fat) or a portion control diet (29% total energy from fat) for six weeks by overweight and obese individuals, oral fatty acid sensitivity was significantly increased. Although the percentage energy from fat was higher in the portion control group, there were no significant differences between groups in the grams of fat consumed, suggesting that the grams of fat consumed is an important factor regarding oral fatty acid sensitivity, rather than the percentage energy of fat consumed. Interestingly, significant increases in the ability of subjects to detect small differences in the fat content of custards occurred in the low-fat diet group only. Nevertheless, directional changes were also seen in the portion control group, however these were not significant changes. This suggests that the types of foods consumed over the six-week period may have played a role. For example, subjects following the portion control diet did not change their intake of dairy products, fried foods, desserts or condiments compared to the low-fat diet group, therefore repeated oral exposure to these types of high-fat foods may explain why significant increases in the portion control group's

ability to perceive fat were not found. In addition, perhaps significant changes in preference would have been seen if the intervention was longer, or there was a higher number of subjects in the study. This study also investigated preference shifts over the six week period in the liking of regular- and low-fat foods. We report that there were minimal changes in preference seen over this time (only liking of low-fat cream cheese increased from baseline to week six), which suggests that a six-week period may have been too short to see such changes and further studies will be needed to ascertain if links between changes in oral fatty acid sensitivity and preference for high- and low-fat foods via dietary changes exist. It is also important to mention that no changes in threshold measures were found for sucrose or NaCl from baseline to week six after consumption of either the low-fat or portion control diet, indicating that changes in fat intake may modulate oral fatty acid sensitivity and were not broadly related to increased sensitivity in the taste system or a learning effect of the testing procedure. Given that previous research has found inverse associations between oral fatty acid sensitivity and dietary fat intake, these data have implications regarding the adherence to low-fat diets, as well as their success in an overweight and obese population as prescription of a low-fat diet may not be the most effective method of weight loss for the whole population. For example, an overweight/ obese individual may be sensitive to fatty acids, but less sensitive to carbohydrates/ sugars. If they are instructed to follow a low-fat diet, they will reduce consumption of fats, which normally induce satiety, and concurrently increase consumption of carbohydrates, which may be less effective at inducing satiety in this individual. Therefore, overconsumption of carbohydrates will occur to induce satiety, resulting in excess energy consumption and no weight loss. In contrast, a

moderate-fat diet portion control diet would be more beneficial for a fatty acid sensitive individual as consumption of fat would be permitted. The modest fat content of such a diet would induce satiety, thereby reducing overall energy intake and weight loss would occur. Studies investigating the influence of fatty acid sensitivity on appetite regulation, satiety and weight loss should be a major focus of future research in the area.

7.2 Conclusions

7.2.1 Major findings

- Large inter-individual variation in oral sensitivity to C18:1, C18:2 and C12:0 exists amongst the population, however small intra-individual variation exists, suggesting that oral fatty acid detection thresholds are reproducible and reliable when using ascending forced choice triangle tests. These findings also mirror those found for the five primary taste qualities.

- Stability in the oral fatty acid threshold measurement is not affected by the macronutrient composition of the meal consumed immediately prior to threshold testing. Consumption of a high-fat, low-fat or macronutrient balanced breakfast had no significant effect on a subject's oral detection thresholds for C18:1.

- Oral sensitivity to C18:1 was increased following the consumption of the low-fat (25% total energy from fat) diet and the portion control diet (29% total energy from fat) over a six week period in an overweight/ obese

population. An increase in an individual's oral perception of fats within a food was also seen in the low-fat diet group only. Changes in preference for regular- and low-fat foods were only seen for one type of food (increased liking of the low-fat cream cheese) after consumption of a low-fat and portion control diet over six weeks suggesting that a longer time period may be needed to see definitive changes.

7.3 Future directions

The studies in this thesis have contributed to and extended research of the potential existence and function of oral fatty acid sensitivity in humans. It has detailed that large inter-individual variation exists in regards to oral fatty acid sensitivity, however minor intra-individual variation exists within subjects suggestive of the high test-retest reliability of oral fatty acid detection thresholds. This thesis has also reported that the macronutrient composition of the meal consumed immediately prior to threshold testing does not impact on oral fatty acid threshold measurements. To the best of our knowledge, these differences in oral sensitivity to un-oxidised fatty acids were due to taste cues, not other discriminatory based differences such as odour, texture and visual cues which were minimised to the best of our ability. In order to verify the existence of oral fatty acid sensitivity as a modality in humans, future research will need to focus on putative receptors for fatty acid detection, specifically whether the putative fatty acid receptors in oral tissue reflect oral sensitivity, how fatty acids interact with these receptors at a cellular level and how environmental factors, for example a high-fat diet, influence receptor density and functionality.

Studies herein have highlighted that sensitivity to C18:1 can be modulated over a six week period following exposure to a low-fat diet and a portion control diet, specifically with a reduction in total amount of fat in grams consumed. When the two diet groups were compared, there were no differences in the amount of fat consumed between the groups or in the magnitude of change in oral fatty acid sensitivity. Conversely, there was a trend (P = 0.058) for a difference in fat percentage consumed at week six between groups (low-fat diet group: 24.9% fat, portion control diet group: 29.2% fat). These finding suggest that the grams of fat consumed may be the major potential influencer of oral fatty acid sensitivity in an overweight and obese population rather than percentage of fat consumed. Similarly to previous research, the present study has reported that following a period of restriction in the grams of fat consumed, oral fatty acid sensitivity was increased, however energy intake in both groups was also reduced making it difficult to conclude that energy intake is not also a factor. It is also difficult to conclude whether weight loss is associated with increased oral fatty acid sensitivity as modulation of sensitivity is yet to be shown independent of weight loss. Therefore, future research will need to develop interventions that control for changes in weight loss and energy intake, for example a moderate-fat, high-kilojoule diet compared to a high-fat, low-kilojoule diet, so that changes in sensitivity can be reported without changes in energy intake and weight loss occurring. Additionally, intervention studies investigating the effect of a long-term low-fat diet on satiety responses is also an important next step. Previous research has found that hyposensitive individuals were less satiated by a high-fat meal than hypersensitive individuals (Keast, et al., 2013). Thus, if oral sensitivity can be increased by a low-fat diet, perhaps satiety

responses can also be enhanced, resulting in reduced excess energy consumption and in turn, decreases in body weight and BMI.

Although this study found positive results in that oral fatty acid sensitivity can be increased with fat restriction, it is important to keep in mind that oral fatty acid sensitivity can also potentially be decreased after consumption of a high-fat diet. Stewart et al. (2012) reported that over a four week period, oral sensitivity to C18:1 was significantly decreased after consumption of a high-fat diet (Stewart & Keast, 2012). Interestingly, this change was only noted in the lean subjects and not in the obese subjects. Reasons for this were hypothesised by the authors and it was suggested that the obese population were already consuming a high-fat diet prior to commencement of the study and had potentially already adapted to the high-fat environment, therefore no effect was seen in their oral fatty acid sensitivity (Stewart & Keast, 2012). Adaptation to the high-fat diet or low-fat diet may have included changes in receptor expression in the oral cavity and GI tract and this will be an important area to focus future research on, given that sensitivity to other tastants is genetically determined (Drewnowski, Henderson, & Barratt-Fornell, 1998; Garcia-Bailo, Toguri, Eny, & El-Sohemy, 2009; Guo & Reed, 2001; Keller, et al., 2002; Kim, Breslin, Reed, & Drayna, 2004). Investigating whether receptor expression is altered after long-term consumption of a low-fat diet, whether specific genes can be switched ‘on’ or ‘off’ in response to the environment they are exposed to and how that links to changes in oral fatty acid sensitivity will be important considerations for future research.

Studies one and two of this thesis failed to find any associations between BMI and oral fatty acid sensitivity, however study three did find an association between oral fatty acid sensitivity and BMI. This is reflective of the literature surrounding this area with some studies finding correlations while others find no relationship. Reasons for this may be due to the fact that studies which found a relationship between BMI and sensitivity used an abbreviated screening procedure to determine oral fatty acid sensitivity and grouped subjects into hypersensitive and hyposensitive, whereas studies that found no associations used a complete threshold testing procedure (3-AFC, staircase methodology *etc.*) (Running, et al., 2013; Stewart, et al., 2010; Stewart, Newman, et al., 2011). Variance in oral sensitivity to C18:1 cannot be seen with the grouping method, and therefore relationships between fatty acid sensitivity and BMI may be exaggerated. Perhaps the most important factor is that the development of overweight/ obesity is influenced by many factors, therefore it could be expected that there would only be weak to moderate associations found. Nevertheless, this raises the question of whether there is an association between the oral fatty acid sensitivity and BMI, and future research should be conducted to ascertain whether there is a link and to identify potential implications for reduction of weight and prevention of obesity. While this thesis found that consumption of a low-fat diet will increase an individual's oral sensitivity and in turn decrease BMI, this association may not apply to the whole population. Sensitivity to fatty acids does not only relate to the oral cavity, but also the GI tract. Research has reported that the obese population have a dysfunction in appetite regulation; in other words, their appetitive responses, including hormone release and satiety mechanisms are attenuated in response to fat ingestion and digestion (Stewart, Seimon, et al., 2011).

Conversely, due to the conflicting findings regarding sensitivity and BMI, some overweight or obese individuals may be sensitive to fat in both the oral cavity and GI tract, thus their hormone and satiety responses will be normal when fat is ingested. However, the same individual may be insensitive to other macronutrients for example, carbohydrates or proteins, therefore when these macronutrients are ingested, the satiety response is attenuated. Thus, by restricting the amount of fat consumed in a fat sensitive individual, increases in the amount of carbohydrates or proteins consumed may occur, *i.e.,* excess energy intake, which may in turn lead to continued weight gain. Thus, oral fatty acid sensitivity screening, as well as screening for sensitivity to carbohydrates and proteins, may be an important procedure to ascertain the dietary intervention that will best suit each individual. This information will be useful in designing nutritional interventions targeted at decreasing dietary fat consumption, and perhaps aid in weight loss and weight management.

This thesis did not find any changes in preference immediately after a high-fat meal or after a prolonged period of fat restriction. This suggests that six weeks is long enough to increase oral fatty acid sensitivity, however is not prolonged enough to see adaptive changes in preference. To enable adherence and success of a low-fat diet, preference for low-fat foods needs to be established and is of great importance for future investigations as for changes in habitual diet to be sustained long-term, a change in preference needs to occur.

Understanding the factors which affect excess energy and dietary fat intake as well as fat preference is an essential step in reducing fat intake and consequently obesity. The findings from this thesis add to the growing body of evidence seeking to understand the functional role of oral fatty acid detection in humans. Oral sensitivity to fatty acids is a novel research topic and there are still many gaps in the knowledge base including the role of diet and appetite regulation and genetics, therefore a number of research studies need to be conducted in the future to address these gaps. This thesis has answered some of the many research questions within this field, and in doing so has contributed to the body of evidence surrounding the association between oral fatty acid sensitivity and diet, emphasising the importance of continued research in this area. As detailed throughout this thesis, oral fatty acid sensitivity seems to mimic characteristics of the primary tastes qualities in its reliability and reproducibility and plays an important role in the detection of fat within the oral cavity and regulation of fat intake. As such, fatty acid sensing within the oral cavity, fat intake regulation and appetite regulation all seem to be associated and may manifest in body weight regulation and importantly, predisposition to obesity.

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Appendix A

Screening form

Screening form

Subject ID_____

Source of study_____

Date_____

1. D.O.B_____ Age_____ (include 18-75 years)
2. Male or female_____
3. Estimated weight (kg)_____
4. Estimated height (metres)_____
5. Estimated BMI (kg/m^2)_____

(include BMI>20 (kg/m^2)

6. Do you currently smoke? Yes / No
7. If no to question 6, were you previously a smoker?
8. If yes to question 7, when did you stop smoking?_____
9. Have you ever been diagnosed with a heart condition? Yes/No

If yes, provide details

_____

_____

10. Do you have any other medical conditions? Yes/No

If yes, provide details

_____

11. Are you taking any prescription medications on a regular basis? Yes / No

If yes, fill in details below.

Medication name	**Reason**
Eg Lipitor	High cholesterol

12. Are you taking any non prescription medications on a regular basis? Yes / No

If yes, fill in details below

Medication name	**Reason**
Eg Panadol	Headache

13. Do you have any food allergies or food intolerances? Yes / No

If yes, provide details_____

14. Will you be able to make dietary changes for 6 weeks? Yes / No

(eg reduce fat, 2 serves of fruit/day, 5 serves veg/day, reduce takeaway food and restaurant meals)

15. Will you be able to do moderate exercise for 30 minutes per day for 6 weeks? Yes / No

(eg walking or swimming)

16. Will you be able to taste test a variety of different foods including milk, custard, mousse, cream cheese and yoghurt? Yes / No

17. Are you able to attend Deakin University at the Burwood Campus for two appointments? Yes / No

Subject eligible. Yes / No

If no, provide reason

_____

Plan

Appointment booked for WAT study. Yes/No
If no, explain plan

_____
_____

Date of
appointment:_____

Car park booked. Yes/No Research Carpark 1 or 2

Date and time of reminder_____

Date paperwork sent:_____

Telephone call
reminder:_____

Appendix B

Detection threshold form

Threshold form

Subject ID:

Session #:

DOB:

Height:

Weight:

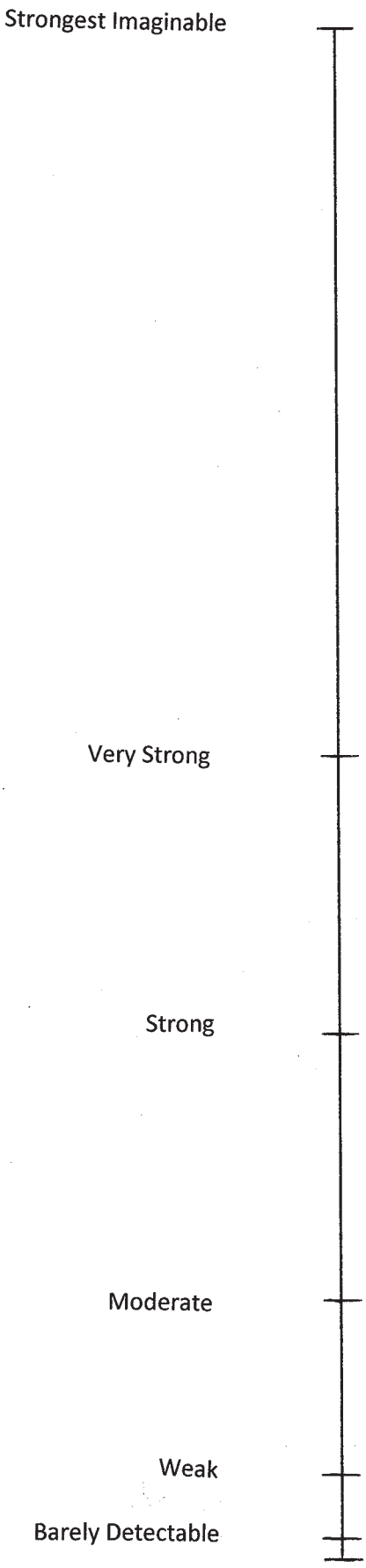
Concentrations: Oleic acid

CONCENTRATION	ATTEMPTS (X = incorrect, ✓ = correct)			
0.02mM				
0.06mM				
1.0mM				
1.4mM				
2.0mM				
2.8mM				
3.8mM				
5.0mM				
6.4mM				
8mM				
9.8mM				
12mM				

Appendix C

gLMS used to determine taste sensitivity to PROP

gLMS Scale for PROP Taste Sensitivity



Appendix D

Fat ranking task answer sheet

Subject ID:

Week:

Fat ranking task answer sheet

Please taste each of the custard samples individually, and then place them in order of perceived fattiness (lowest amount of fat to the highest amount of fat).

Please write down the code of each sample in the order you think they belong:

1. _____ (sample with the **LEAST** amount of fat)

2. _____

3. _____

4. _____ (sample with the **MOST** amount of fat)

Appendix E

Answer sheets for liking of regular-fat and low-fat foods

Subject ID_____

Sensory testing answer sheet (biscuits and cream cheese)

For each set of samples, please taste and rate each sample individually. Take care that the sample you are tasting matches the three digit code

Sample # 429

Please taste the following sample and place a mark in the box which you feel best describes how much you like the product:

☐	☐	☐	☐	☐	☐	☐	☐	☐
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Sample # 853

Please taste the following sample and place a mark in the box which you feel best describes how much you like the product:

☐	☐	☐	☐	☐	☐	☐	☐	☐
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Subject ID_____

Sensory testing answer sheet (vanilla yoghurt)

For each set of samples, please taste and rate each sample individually. Take care that the sample you are tasting matches the three digit code

Sample # 187

Please taste the following sample and place a mark in the box which you feel best describes how much you like the product:

☐	☐	☐	☐	☐	☐	☐	☐	☐
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Sample # 355

Please taste the following sample and place a mark in the box which you feel best describes how much you like the product:

☐	☐	☐	☐	☐	☐	☐	☐	☐
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Subject ID_____

Sensory testing answer sheet (chocolate mousse)

For each set of samples, please taste and rate each sample individually. Take care that the sample you are tasting matches the three digit code

Sample # 631

Please taste the following sample and place a mark in the box which you feel best describes how much you like the product:

☐	☐	☐	☐	☐	☐	☐	☐	☐
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Sample # 948

Please taste the following sample and place a mark in the box which you feel best describes how much you like the product:

☐	☐	☐	☐	☐	☐	☐	☐	☐
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Appendix F

Food record form

Food Record

24 HR RECORD

Instructions:

- Be as specific as possible with brand names and amounts
- Please see example below

Meal	Time	Food/drink	Quantity
B/F	7am	Kelloggs Cornflakes	1 cup
		Milk – Pura light start	½ cup
		Sugar – white, table sugar	2 tsp
		Tea – tea bag, weak	200 ml
		Milk – Skinny milk	2 tblsp

Day and Date Recorded _____ __/__/_____

Meal	Time	Food/drink	Quantity

Appendix G

Food frequency questionnaire

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DEAKIN

Food Frequency Questionnaire

Student ID Number: | | | | | | | | | |

Background

This questionnaire is designed to estimate your **usual** pattern of food intake by providing us 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'.

Example	Average number of times consumed in the last month								
Please answer the following questions by putting a cross in the box in the column that applies to you for that item.	Never, or less than once a month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	Once per day	2-3 times per day	4-5 times per day	6+ times per day
Please cross only one box per row									
Pineapple	☐	☐	☐	☒	☐	☐	☐	☐	☐

Completion of this questionnaire is voluntary

Please read this page before completing the questionnaire

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 '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.

	Average number of times consumed in the last month								
	Never, or less than once a month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	Once per day	2-3 times per day	4-5 times per day	6+ times per day
Dairy Foods									
Flavoured milk drink (e.g. milkshake, iced coffee, hot chocolate	☐	☐	☐	☐	☐	☐	☐	☐	☐
Milk as a drink	☐	☐	☐	☐	☐	☐	☐	☐	☐
Milk on breakfast cereals	☐	☐	☐	☐	☐	☐	☐	☐	☐
Milk in hot beverages (e.g. in coffee, tea)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cream or sour cream	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ice-cream	☐	☐	☐	☐	☐	☐	☐	☐	☐
Yoghurt, plain or flavoured (including fromage frais)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cottage or ricotta cheese	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cheddar and other cheeses	☐	☐	☐	☐	☐	☐	☐	☐	☐
Bread and Cereal Foods									
White bread, toast or rolls	☐	☐	☐	☐	☐	☐	☐	☐	☐
Wholemeal/mixed grain bread, toast or rolls	☐	☐	☐	☐	☐	☐	☐	☐	☐
English muffin, bagel or crumpet	☐	☐	☐	☐	☐	☐	☐	☐	☐
Dry or savoury biscuits, crispbread, crackers	☐	☐	☐	☐	☐	☐	☐	☐	☐
Muesli	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cooked porridge	☐	☐	☐	☐	☐	☐	☐	☐	☐
Breakfast cereal	☐	☐	☐	☐	☐	☐	☐	☐	☐
Rice (including white or brown)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pasta (including filled), noodles	☐	☐	☐	☐	☐	☐	☐	☐	☐
Meat, Fish, Eggs									
Mince dishes (e.g. rissoles, meat loaf)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Mixed dishes with beef, veal (e.g casserole, stir-fry)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Beef, veal - roast, chop or steak	☐	☐	☐	☐	☐	☐	☐	☐	☐
Mixed dishes with lamb (e.g casserole, stir fry)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lamb - roast, chop or steak	☐	☐	☐	☐	☐	☐	☐	☐	☐
Mixed dishes with pork (e.g casserole, stir fry)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pork - roast, chop or steak	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sausage, frankfurter	☐	☐	☐	☐	☐	☐	☐	☐	☐
Bacon	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ham	☐	☐	☐	☐	☐	☐	☐	☐	☐
Luncheon meats, salami	☐	☐	☐	☐	☐	☐	☐	☐	☐
Liver (including pate)	☐	☐	☐	☐	☐	☐	☐	☐	☐

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6. When cooking, which of the following oils/fats do you use?

☐ Olive oil ☐ Margarine

☐ Canola oil ☐ Dairy blend

☐ Vegetable oil ☐ Lard or dripping

☐ Butter

7. How often (do you/does) 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.

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.

	Never/ Rarely	Sometimes	Usually
Light cream	☐	☐	☐
Sour light cream	☐	☐	☐
Low/reduced fat ice-cream	☐	☐	☐
Low/reduced fat cheddar-type cheese	☐	☐	☐
Low/reduced oil salad dressing	☐	☐	☐
Low/reduced fat spreads	☐	☐	☐

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
- ☐ 6 serves or more
- ☐ 2-3 serves
- ☐ Don't eat vegetables
- ☐ 4-5 serves

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
- ☐ 6 serves or more
- ☐ 2-3 serves
- ☐ Don't eat fruit
- ☐ 4-5 serves

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Average number of times consumed in the last month

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.	Never, or less than once a month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	Once per day	2-3 times per day	4-5 times per day	6+ times per day
Other offal (e.g kidneys)									
Mixed dishes with chicken, turkey, duck (e.g casserole, stir-fry)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chicken, turkey, duck - roast, steamed, BBQ	☐	☐	☐	☐	☐	☐	☐	☐	☐
Canned tuna, salmon, sardines	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fish, steamed, baked, grilled	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fish, fried	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other seafood (e.g prawns)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Egg	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sweets, Baked Goods and Snacks									
Cakes, sweet muffins, scones or pikelets	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sweet pies or sweet pastries	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other puddings or desserts	☐	☐	☐	☐	☐	☐	☐	☐	☐
Plain sweet biscuits	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cream, chocolate biscuits	☐	☐	☐	☐	☐	☐	☐	☐	☐
Meat pie, sausage roll or other savoury pastries	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pizza	☐	☐	☐	☐	☐	☐	☐	☐	☐
Hamburger	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chocolate (including chocolate bars e.g Mars bars	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other confectionery	☐	☐	☐	☐	☐	☐	☐	☐	☐
Jam, marmalade, syrup or honey	☐	☐	☐	☐	☐	☐	☐	☐	☐
Peanut butter, other nut spreads	☐	☐	☐	☐	☐	☐	☐	☐	☐
Vegemite, Marmite, Promite	☐	☐	☐	☐	☐	☐	☐	☐	☐
Nuts	☐	☐	☐	☐	☐	☐	☐	☐	☐
Potato chips, corn chips, Twisties, etc	☐	☐	☐	☐	☐	☐	☐	☐	☐
Dressings									
Oil and vinegar dressing	☐	☐	☐	☐	☐	☐	☐	☐	☐
Mayonnaise or other creamy dressing	☐	☐	☐	☐	☐	☐	☐	☐	☐
Non-dairy Beverages									
Fruit juice	☐	☐	☐	☐	☐	☐	☐	☐	☐
Vegetable, tomato juice	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fruit juice drink or fruit drink	☐	☐	☐	☐	☐	☐	☐	☐	☐
Low-joule cordial	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cordial	☐	☐	☐	☐	☐	☐	☐	☐	☐

Average number of times consumed in the last month

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.

	Never, or less than once a month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	Once per day	2-3 times per day	4-5 times per day	6+ times per day
Low-joule soft drink	☐	☐	☐	☐	☐	☐	☐	☐	☐
Soft drinks (including flavoured mineral water)	☐	☐	☐	☐	☐	☐	☐	☐	☐
Water (including unflavoured mineral water, soda water, tap water	☐	☐	☐	☐	☐	☐	☐	☐	☐
Coffee	☐	☐	☐	☐	☐	☐	☐	☐	☐
Tea	☐	☐	☐	☐	☐	☐	☐	☐	☐
Soy beverages	☐	☐	☐	☐	☐	☐	☐	☐	☐
Beer - low alcohol	☐	☐	☐	☐	☐	☐	☐	☐	☐
Beer -ordinary	☐	☐	☐	☐	☐	☐	☐	☐	☐
Red wine	☐	☐	☐	☐	☐	☐	☐	☐	☐
White wine or champagne/sparkling wine	☐	☐	☐	☐	☐	☐	☐	☐	☐
Wine cooler	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sherry or port	☐	☐	☐	☐	☐	☐	☐	☐	☐
Spirits, liqueurs	☐	☐	☐	☐	☐	☐	☐	☐	☐
Vegetables (including frozen and tinned) Green/mixed salad (including lettuce, tomato, etc)	☐	☐	☐	☐	☐	☐	☐	☐	☐
In a sandwich	☐	☐	☐	☐	☐	☐	☐	☐	☐
As a side salad/with a main meal	☐	☐	☐	☐	☐	☐	☐	☐	☐
Stir-fried or mixed vegetables	☐	☐	☐	☐	☐	☐	☐	☐	☐
Vegetable casserole	☐	☐	☐	☐	☐	☐	☐	☐	☐
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	☐	☐	☐	☐	☐	☐	☐	☐	☐
Silverbeet, spinach	☐	☐	☐	☐	☐	☐	☐	☐	☐
Broccoli	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cauliflower	☐	☐	☐	☐	☐	☐	☐	☐	☐
Brussel sprouts, cabbage, coleslaw	☐	☐	☐	☐	☐	☐	☐	☐	☐
Carrots	☐	☐	☐	☐	☐	☐	☐	☐	☐

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Average number of times consumed in the last month

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.

	Never, or less than once a month	1-3 times per month	Once per week	2-4 times per week	5-6 times per week	Once per day	2-3 times per day	4-5 times per day	6+ times per day
Zucchini, eggplant, squash	☐	☐	☐	☐	☐	☐	☐	☐	☐
Capsicum	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sweetcorn, corn on the cob	☐	☐	☐	☐	☐	☐	☐	☐	☐
Mushrooms	☐	☐	☐	☐	☐	☐	☐	☐	☐
Tomatoes	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lettuce	☐	☐	☐	☐	☐	☐	☐	☐	☐
Celery, cucumber	☐	☐	☐	☐	☐	☐	☐	☐	☐
Onion or leeks	☐	☐	☐	☐	☐	☐	☐	☐	☐
Soybeans, tofu	☐	☐	☐	☐	☐	☐	☐	☐	☐
Baked beans	☐	☐	☐	☐	☐	☐	☐	☐	☐
Other beans, lentils	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fruits (including dried, frozen and tinned)									
Apple or pear	☐	☐	☐	☐	☐	☐	☐	☐	☐
Orange, mandarin or grapefruit	☐	☐	☐	☐	☐	☐	☐	☐	☐
Banana	☐	☐	☐	☐	☐	☐	☐	☐	☐
Peach, nectarine, plum or apricot	☐	☐	☐	☐	☐	☐	☐	☐	☐
Mango or paw-paw	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pineapple	☐	☐	☐	☐	☐	☐	☐	☐	☐
Grapes or berries	☐	☐	☐	☐	☐	☐	☐	☐	☐
Melon (e.g watermelon, rockmelon, honeydew melon	☐	☐	☐	☐	☐	☐	☐	☐	☐
Vitamin and Mineral Supplements (including tablets, capsules or drops)									
Multi vitamin with iron or other minerals	☐	☐	☐	☐	☐	☐	☐	☐	☐
Multi vitamin	☐	☐	☐	☐	☐	☐	☐	☐	☐
Vitamin A	☐	☐	☐	☐	☐	☐	☐	☐	☐
Vitamin B	☐	☐	☐	☐	☐	☐	☐	☐	☐
Vitamin C	☐	☐	☐	☐	☐	☐	☐	☐	☐
Vitamin E	☐	☐	☐	☐	☐	☐	☐	☐	☐
B-carotene	☐	☐	☐	☐	☐	☐	☐	☐	☐
Calcium	☐	☐	☐	☐	☐	☐	☐	☐	☐
Folic Acid/Folate	☐	☐	☐	☐	☐	☐	☐	☐	☐
Iron	☐	☐	☐	☐	☐	☐	☐	☐	☐
Zinc	☐	☐	☐	☐	☐	☐	☐	☐	☐

Appendix H

Recipes for frittatas baked in study two

Balanced frittata (equal proportions of carbohydrate, fat and protein)

8 eggs

1 cup reduced fat milk

300g canned corn, drained

1 cup of frozen peas

1 cup of shaved ham

2 tomatoes

2 carrots

2 medium potatoes

1.5 cups spinach

¾ cup low fat cheddar cheese

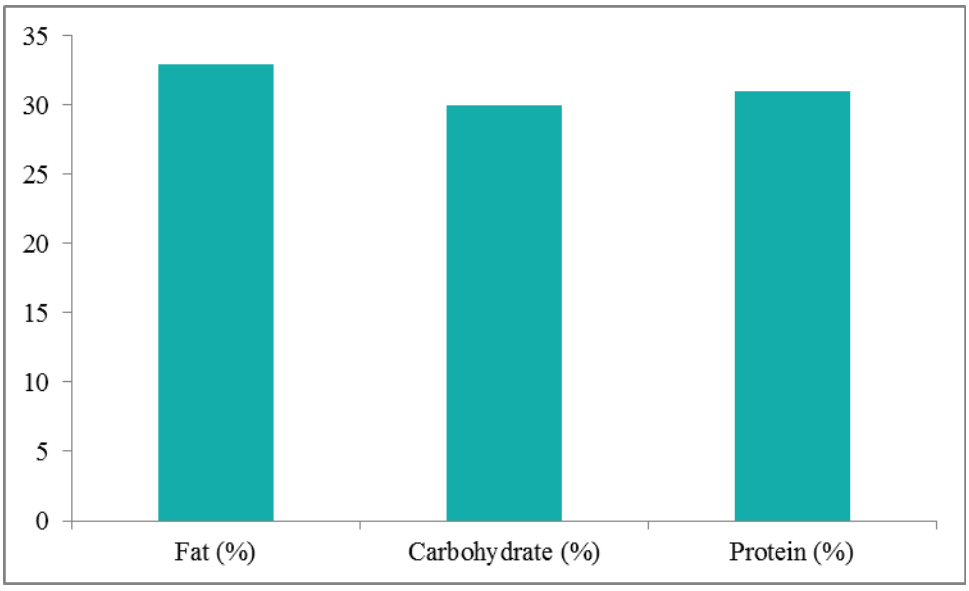
1 medium onion

1 clove garlic

Salt

Pepper

Macronutrient proportions for the balanced frittata



High-fat frittata

9 eggs

¾ cup thickened cream

1 tbs butter

1 large onion

4 rashers of bacon

3 large potatoes

1.5 cups regular cheddar cheese

3 tomatoes

2 tbs olive oil

1 clove garlic

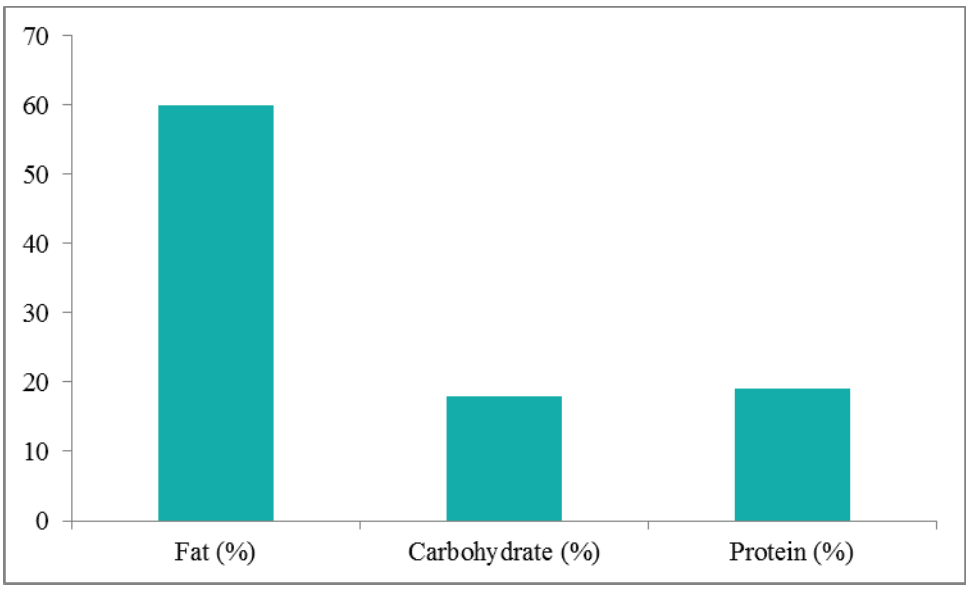
½ cup plain flour

¾ sweet potato, chopped

Salt

Pepper

Macronutrient proportions for the high-fat frittata



Low fat frittata

4 eggs

6 egg whites

1 medium onion

3 medium potatoes

1.25 cups low fat cottage cheese

2 tomatoes

1 clove garlic

½ cup skim milk

1 cup sweet potato, chopped

½ dry macaroni pasta

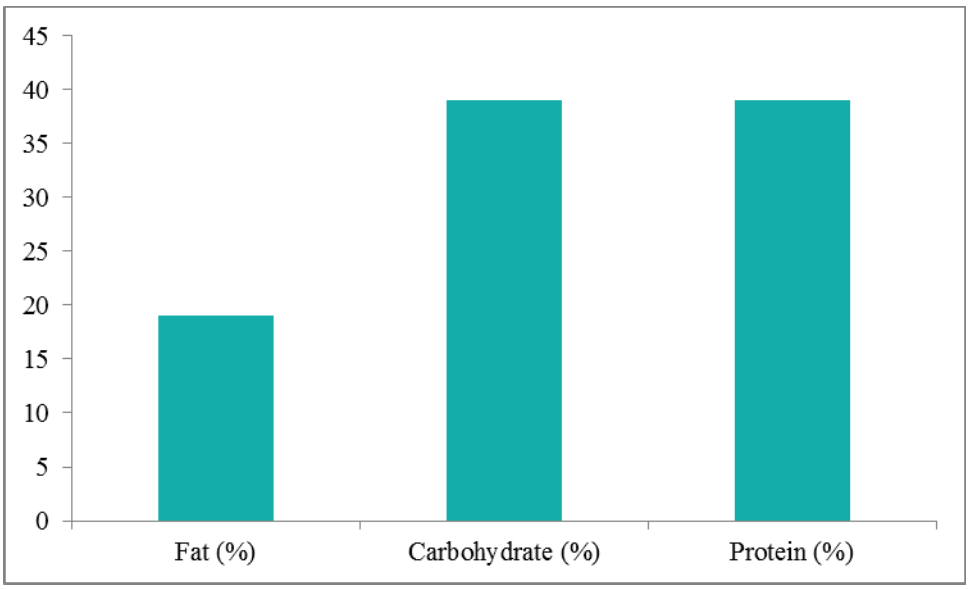
100g canned lentils, drained

¼ low fat cheddar cheese

Salt

Pepper

Macronutrient proportions for the low-fat frittata



Appendix I

Diet booklet given to subjects following the low-fat diet in study three

Deakin University

WAT Study

(Weight Loss and Taste Study)

Diet & Exercise Information Booklet

WAT Diet
(6 weeks)

This diet is a weight loss diet designed to:

Increase

Fruits
Vegetables

Reduce

Full fat dairy products
Fatty meats
Baked products
High fat spreads
Calories/kilojoules

You will be required to follow this diet for 6 weeks.

The following summarises the diet:

Food Group	Daily allowance
Fruit	At least 2 serves/ day
Vegetables	At least 5 serves/ day
Low-fat dairy	3 serves/ day
Protein (fish, chicken, legumes, red meat)	Approximately 1 serve/ day
Fats (mono- and poly-unsaturated)	Maximum 3 serves/ day

FRUIT & FRUIT JUICE

We are asking you to make sure you have **at least 2 serves** of fruit or fruit juice per day.

1 serve is equivalent to any one of the following:

- 1 medium piece of fruit (e.g. 1 banana, 1 orange, 1 apple, 1 pear)
- 1 medium tomato
- ¼ cup dried fruit (e.g. apricots, sultanas) **no more than 1 serve per day**
- ½ cup fresh fruit (e.g. grapes, melon strawberries),
- ½ cup canned fruit but **no more than 1 serve per day (due to the high energy content)**
- ½ cup per day of fruit juice. N more than **1 serve per day** (due to the high energy content)

DAIRY

We are asking you to make sure you have **3 serves** of dairy per day. Milk and yoghurt must be **skim** or **no fat** and cheese must be as listed below.

1 serve is equivalent to any one of the following:

- 1 cup (200ml) of **skim** milk (Physical No Fat, Pura Tone No Fat, Skinny Milk)
- 1 tub (200g) of **skim** yoghurt (Jalna Fat Free, Tamar Valley No Fat, Nestle Diet No Fat, Ski No Fat, Vaalia No Fat, Yoplait No Fat)
- 1/2 cup of **low fat** cottage/ricotta cheese (Bulla Low Fat Cottage Cheese, Coles Farmland Low Fat Cottage Cheese, Weight Watchers Cottage Cheese)

Note - **Cheddar cheese** must be limited to 2 serves of 40g (2 slices) per week and must be the lowest fat varieties (<15% fat e.g. Bega Super Lights, Bega Super Slim, Devondale Seven, Farmland Extra Lights, Kraft Extra Light, Kraft Free, Weight Watchers reduced fat, Coon Extra Light)

VEGETABLES

We are asking you to make sure you have <u>at least</u> 5 serves of vegetables per day.

1 serve is equivalent to any one of the following:

- 1 cup raw leafy vegetables (e.g. lettuce, spinach, cabbage)
- ½ cup cooked vegetables (e.g. corn, cauliflower, pumpkin, sweet potato, carrot, beans, peas, zucchini, leek, mushroom)
- 1 medium potato (preferably whole baked)

PROTEIN FOODS

Try to limit the amount of fatty red meats you consume. Try and substitute these with lean alternatives, for example, fish or chicken. We recommend you have **<u>1-2 serves</u>** per day.

1 serve is equivalent to any one of the following:

- 65–100g cooked meat or chicken (½ cup lean mince, 2 small chops, 2 slices of roast meat)
- ½ cup cooked dried or canned beans, lentils, chick peas or split peas
- 80–100g fish fillet

FATS AND OILS

On this diet you are allowed a **maximum** of **3 serves of fat per day**.

1 serve is equivalent to any of the following:

- 1 tsp of monounsaturated (Canola or Olive Oil) or polyunsaturated oil (but not blended vegetable oils)
- 1 tsp of regular fat margarine
- 2 tsp of reduced fat margarine
- 2 tsp of Avocado

Some Recommended brands to use -

Reduced Fat Margarines

- Flora Light Margarine
- Meadow Lea Light Margarine
- Bertolli Spread light

Please note that these lower fat margarines are not suitable for cooking.

BREAD & CEREALS

We will ask you to have a **maximum of 5 serves** per day. This group includes pasta, rice, wholegrain bread, breakfast cereals etc.

1 serve is equivalent to any of the following:

- 2 slices of bread
- 1 medium bread roll
- 1 cup cooked rice, pasta, noodles
- 1 cup porridge
- 1 cup breakfast cereal flakes
- ½ cup muesli

REDUCING YOUR KILOJOULE INTAKE

Choose mainly plant-based foods such as bread, cereals, rice, pasta, vegetables, fruits and legumes. These foods are high in fibre, naturally low in fat, rich in nutrients and help to satisfy hunger

Preferred cold meats / sandwich fillings

- Fresh chicken or fresh turkey (with cranberry sauce)
- Tuna or salmon in spring water
- Salad
- Fresh roast beef (with pepper), lamb (with mint sauce) or pork (with apple sauce)

Preferred cereals / breads

- Wholemeal or multi grain bread
- Wholegrain cereals (e.g. untoasted muesli, Oats, Kellogg's Mini-wheats, Sanitarium Lite Bix, Just Right)

Preferred Milk and Milk Products

- Skim milk (Physical No Fat, Pura Tone No Fat, Skinny Milk,)
- Skim yoghurt (Yoplait no fat, Nestle diet no fat)

Preferred Cheeses

- Reduced fat ricotta (e.g. Pantalica light, Perfect Italiano Smooth light ricotta cheese)
- Reduced fat cottage (e.g. Bulla Low Fat, Weight Watchers, Farmland Low Fat)

Preferred Dressings

- Vinegar (balsamic, white, red wine etc)
- Lemon juice

Preferred Alcohol (if any)

Alcohol is high in kilojoules (energy) so often contributes to excess weight.
Alcohol should be limited when trying to lose weight.
On this diet try and limit alcohol to a **maximum of 4 standard drinks per week.**

<u>Hints for decreasing alcohol intake:</u>

- Try alternating alcoholic drinks with water
- Swap to low alcohol or 'light' beer or wine
- Mix drinks with low kilo joule soft drinks such as diet cola
- Use only half measures of spirits in mixed drinks
- Put your drink down between sips, talk more and drink less

SOME FOOD FOR THOUGHT...

BEVERAGES

TRY	AVOID
Water	Fruit juice (maximum of ½ cup per day)
Tea, coffee with skim milk and an artificial sweetener	Sports drinks (Powerade etc)
Diet soft drinks and cordials	Flavoured milks
Plain mineral or soda water	Flavoured soft drinks, mineral waters and cordials
	Alcoholic drinks

CONDIMENTS, SWEETENING AGENTS & FLAVOURINGS

TRY	AVOID
Sugar	Commercial mayonnaise and salad dressings
Honey, jam	Ordinary peanut butter
Spices, herbs	Butter
Vinegar	Tartare sauce
Apple or cranberry sauce	Parmesan cheese
Mint sauce	
Vegemite	
Equal sweetener	
LOW FAT sauces and dressings	

TAKE AWAY FOODS

TRY	AVOID
Grilled fish	Hamburgers
Sliced roast meat	Fried and BBQ chicken
Salads	Pizza
Fruit	Indian, Thai, Italian, Chinese, Vietnamese take away
Homemade pizzas	Battered fish and chips

SNACKS

TRY	AVOID
Raw or cooked vegetables	Savoury biscuits (Shapes, Savoys etc)
Unsalted popcorn	Potato crisps
Raisin bread	Sweet biscuits/ cookies
Wholegrain breakfast cereals	Dips
Toasted muffins and crumpets	Baked goods (cake, pies, pasties etc)
Rice cakes or low fat rice crackers	Nuts
No fat yoghurt	
Diet soft drinks	
Fresh or tinned fruit	
Low fat milkshakes or fruit smoothies	
Grainy breads and rolls	

FRESH FOOD SUGGESTIONS AND COOKING IDEAS

Barbeques

- Marinate skinless chicken breast fillets or lean meats in fruit juice or wine before grilling
- Bake fish fillets or kebabs in foils with seasonings and lemon juice
- Make kebabs using lean meat and vegetable chunks
- Pre-cook whole potatoes in the microwave or oven, then crisp on the BBQ

Cooked Vegetables

- Add a squeeze of lemon or ground black pepper to freshly steamed or microwave vegetables
- Stir fry vegetables in small amounts of oil or use water
- Add skim yoghurt to jacket potatoes instead of sour cream

Dinner

- Have lots of vegetables, and use smaller portions of lean meat, chicken or fish
- Use low fat ricotta cheese in cheese dishes, with a sprinkle of parmesan cheese for flavour

Frying foods

- Use a non stick fry pan
- Brush the base of the pan with oil such as canola, sunflower, olive or peanut oil, to prevent food from sticking
- Microwave vegetables and then brown them under the griller

Soups and sauces

- Use evaporated skim milk for ‘cream’ soups
- Use pureed vegetables to thicken sauces
- Use wholegrains, barley, or lentils to thicken casseroles or stews

Lunch

- Fill sandwiches with lots of salad vegetables and a small serving of lean meat, skinless chicken, canned fish (in spring water), hummus or a low fat cheese such as cottage cheese
- Toasted sandwiches. Try filling with baked beans or lean ham and asparagus
- Have cooked vegies left over from dinner the night before
- Hard boiled eggs (with pepper, curry, mustard powder or chilli powder)
- For variety, try fresh fruit salad and skim yoghurt for lunch

Pasta

- Serve pasta with a sauce that has plenty of vegetables (eg tomato based)
- Limit creamy and cheese based pasta sauces

Roast Dinner

- Select a lean leg of lamb, beef, or pork – trim off any fat
- Roast a chicken and don't eat the skin
- Cook in a roasting pan with a little water or wine
- Brush or spray vegetables with oil such as canola, sunflower or olive oils and bake in a separate pan

Toast

- Spread peanut butter thinly and limit other higher fat toppings such as cheese and cheese spreads
- Spread with ricotta or cottage cheese, marmalade, honey, chutney, mustard or top with a small serve of baked beans, spaghetti or fresh tomato

USEFUL TIPS:

- Prepare extra vegetables or salads at dinner time for use at lunch the following day
- Cut and prepare fruit at the beginning of the day so that it is easily available to eat later that day
- Be creative - Use different sauces, herbs and spices to enhance the flavour of food eg.

bay leaf	brandy	chives
cinnamon	cloves	curry
dill	garlic	ginger
mint	dry mustard	paprika
nutmeg	onions	rosemary
parsley	pepper	lemon juice
sugar	thyme	rum
vanilla	wine	sage
vinegar		

- Use smaller serving dishes at meal times to help reduce portion sizes
- Eat your meal slowly so your body has a chance to register that you are full. If you eat quickly then you are likely to consume more energy than you actually need
- Hunger is a sign that the diet is actually working!
- If you find biscuits, lollies, cakes, soft drinks etc irresistible, then don't have any of these foods at home. If they are not there, then you won't eat them. Set up your home environment so you can only eat healthy foods.

EXAMPLE OF DAILY MEAL PLAN

BREAKFAST:	½ cup muesli ½ cup skim milk 200g low-fat yogurt
MORNING SNACK:	2 rice cakes with vegemite 1 carrot (cut into sticks)
LUNCH:	2 slices wholegrain bread (toasted sandwich) 1 teaspoon light margarine 150g baked beans 1 glass water
AFTERNOON SNACK:	1 piece of fresh fruit or 30g sultanas
DINNER:	150g Satay chicken stir fry (with vegetables: capsicum, onion, snow peas, carrot etc) ½ cup brown rice
DESSERT:	½ cup low fat custard 1 piece of fruit

TIPS FOR DINING OUT

Modify meals to suit you

Here's where you have to take some responsibility for your own healthy eating and speak up by:

- Checking the ingredients and preparation method of meals with your waiter.
- Asking to replace different ingredients with healthier options. For example: vegetables instead of chips, skim milk rather than full cream.
- Requesting sauces or dressings on the side, that way you can control the quantity on your plate.
- Not being swayed by pushy waiters or jibes from your fellow dinners; just order what you want.

Enjoy, don't indulge

It's all about portions. Keep your meal sizes small by:

- Have light meals and snacks during the day if you know you are going to have a restaurant meal at night.
- Sharing dishes to keep portions sizes down or ordering two entrees rather than one entree and a main meal.
- Making sure you have a booking so you can go straight to your table rather than wait at the bar.
- Have water with your meal rather than soft drink, juices or alcohol

Know what is good and bad

Good:

- Balsamic vinegar dressings, salsa, cocktail, soy or tomato sauce, mustard and lemon.
- Foods which are described as steamed, fresh, baked, roasted, poached, lightly sautéed or stir-fried.
- Lean steaks such as tenderloin, club and sirloin.
- Seafood; even the fattest fishes have less fat than the leanest meats.

The bad:

- Creamy soups, stick to clear ones instead.
- Fatty steak cuts such as rib eye, porterhouse, ribs and brisket.
- Quiches and omelettes if made with cheese.
- Antipastos.

HEALTHY MENU SUGGESTIONS...

Breakfast

- Opt for muesli and cereals over sticky danishes and croissants.
- Ask for eggs poached or boiled.
- Choose brown or wholegrain toast over white.
- Pancakes are better topped with jam, low-fat yoghurt or fruit rather than maple syrup and ice-cream.
- Most muffins are actually high in fat. Look for the low-fat varieties or just skip them altogether.
- English muffins and bagels are good choices, but ask for spreads on the side.

Lunch

- Sandwiches on wholegrain or brown bread
- Say no to butter, mayonnaise, gravy and cheese.
- Choose turkey or ham
- In salads skip the croutons and bacon bits and go for tuna or chickpeas instead.

Dessert

- Fruit is your best option. Sorbets and yoghurts are also good.
- If you absolutely can't resist a chocolate mud cake, share with a friend so you don't eat more than your share. Say no to whipped cream

LABEL READING

Food labels follow certain rules set down by law and they contain a lot of information. Reading labels can tell you a lot about the food you're eating (see attached handout).

INGREDIENTS

The ingredients on the label are listed in order of quantity. This means there is more of the ingredients higher on the list compared to those lower down the list. Try to avoid food with high amounts of fat. A low fat food generally has less than 10 grams of fat per 100 grams.

EXERCISE GUIDELINES

As part of a weight loss regime it is also very important to include some physical activity. It will be best if you start exercising slowly, but please make sure you have been given clearance by your doctor before you start.

Your aim by the end of the study will have completed 30 minutes of exercise every day, but to start with you should focus on whatever you can comfortably achieve and you may even want to begin at a lower intensity.

The most important thing with this exercise program is that you are consistent. Your exercise program should be continued daily and this will allow you to increase your duration and intensity of the exercise and your fitness levels will begin to rise. When increasing your exercise you can start by either increasing the duration you are exercising but keeping the intensity the same, or increase your intensity and keep your duration the same.

If required, the level of exercise that you do must be approved by your doctor. Walking will be the best way to start your program. A general guideline may be to start with 30 minutes of very low intensity walking (see attached 'physical activity' sheet), and this can be broken up into intervals if needed. Once you can comfortably walk for 30 minutes at a time, try to gradually increase the intensity. When you become comfortable with walking for 30 minutes, maybe increase your duration to 40 minutes and when you become comfortable at each level, increase either the duration or intensity of the exercise.

It is important to remember your body will not improve its performance immediately and that the increase in duration and intensity should be gradual. Please make sure that you do not do anymore than feels comfortable!

SOME POINTS TO REMEMBER:

- Weight loss is not simple. It takes effort and time
- You must use up more energy through physical activity and consume less kilojoules (energy) from food and drinks
- Weight loss must be gradual to ensure it is fat loss not muscle or water
- Healthy eating is not dieting, it's everyday eating
- Don't weigh yourself every day. Weigh yourself no more than once a week as your weight is likely to fluctuate daily
- Limit high kilojoule foods and drinks
    - Foods to enjoy include bread, cereals, rice, pasta, vegetables, fruits, legumes, lean meats, fish and skim dairy products
- Reduce saturated fat
    - This will help reduce your overall energy intake as well as help reduce your risk of heart disease
- Be more physically active
    - All physical activity helps burn up extra kilos
- Reward yourself (e.g. movies, not food) when you have maintained a lifestyle change for a certain length of time
- Take time over your meals, enjoy your food, savour the taste of each mouthful, don't be rushed by others and eat to a comfortable level of fullness
- Try not to buy tempting high kilojoule foods like chocolates and pastries but look for healthier alternatives
- Think positively
- Get support from family and friends-everyone can enjoy healthy eating

Good luck ☺

SUITABLE SNACKS
ON THE WAT DIET

Although the WAT diet is a weight loss diet, suitable snacks can be enjoyed.

Weight loss always comes down to reducing your energy intake so you take in less energy than you expend (exercise). So you are in negative energy balance.

Since all foods are a source of energy (kilojoules/calories), the first rule of thumb is that if you are not hungry it is obviously better if you do not eat anything.

However, if you are hungry then choosing a suitable snack can prevent over eating or binge eating later on in the day.

Common snack foods are high in energy and/or salt and/or fat e.g. potato chips, corn chips, sweet biscuits, chocolates, cakes, pastries, muffins, chocolate bars, salted nuts, dry biscuits, regular fat cheese, dips, muesli bars, high fat dairy snacks, ice creams, hot chips, potato cakes and dim sims

SUGGESTED SNACK FOODS

- Fresh whole fruit of any type (except avocado), e.g. bananas, apples, oranges, pears, grapes, melon, berries, peaches, pineapple, plums, nectarines, apricots, grapefruits, kiwi fruits, mangoes, mandarins and many more!
- Fresh fruit salad
- Raw carrot and celery sticks and low fat/low salt dip
- Cherry tomatoes
- Rice cakes (and jam or vegemite)
- Unsalted popcorn (no butter)
- No Fat yoghurts
- No Fat drinking yoghurt
- Skim milk fruit smoothies
- A bowl of lower fat cereals and skim milk
- Pita bread and low fat/low salt dip

EATING OUT AND TAKE AWAYS ON THE WAT DIET

Because the WAT diet is a weight loss diet, eating out can be difficult. Restaurant meals are often high in fat and salt. If you eat out regularly, it is impossible to be compliant on the WAT diet. However, an occasional meal out should be able to be included as long as you make appropriate choices.

EATING OUT

HINTS

- Ask about any sauces that come with a meal selection. Often they are made with butter and/or cream and must therefore be avoided. Ask for them not to be added.
- Restaurants often add butter to vegetables. Request that no butter be added.
- Ask for salad dressings not to be added unless it is vinegar or lemon juice only.
- Remove any fat from foods served to you (e.g. the fat and skin from chicken and the visible fat from meat).
- Ask for small meals or choose entrée-sized dishes.
- Beware of smorgasbords – you always eat a lot more than you need.
- Chose lean meats and ask for extra vegetables.
- Avoid garlic and herb breads – they are too high in fat.

SUGGESTED MENU CHOICES

- Entrees:
    - Salads with no oil dressings (request balsamic vinegar or lemon juice)
    - Fruit cocktail
    - Oysters or fresh seafood platter (not deep fried)
- Mains:
    - Grilled, baked or steamed fish (not crumbed or fried)
    - Lean meat (no gravy/marinade)
    - Grilled, baked or poached skinless chicken
    - Avoid sauces
    - Pasta with tomato based sauce (not a creamy or cheesy sauce)
- Salads and Vegetables:
    - Use lemon juice or balsamic vinegar dressings (beware home made dressings which are often made with oil)
    - Choose or ask for grilled, boiled or steamed vegetables
    - Steamed potatoes or jacket potatoes (without butter/margarine)
- Rice and breads:
    - Plain steamed or boiled rice (not fried)

    - All plain breads and rolls (without butter/margarine)
- Desserts:
    - Fresh fruit, fruit salad or stewed fruit
    - Baked apples
    - Sorbet or Gelati
    - Reduced fat custards or reduced fat ice cream
- Drinks:
    - Plain, mineral or soda water
    - Diet soft drinks/diet cordials
    - Tea, coffee, cappuccino, latte etc with skim milk
    - Herbal teas

TAKE AWAY

Because the WELL diet is a weight loss diet, getting low fat, low salt take away foods can be difficult (often impossible). Take away foods are usually high in fat and salt. If you get take away foods more than once a week, it is impossible to be compliant on the WELL diet. However, an occasional take away meal should be able to be included as long as you make appropriate choices.

HINTS

- Virtually all take-away foods contain a lot of kilojoules as well as excessively high levels of sodium and so should be avoided.

- Even take away food from "health food stores" fall into the above criteria

SUGGESTED TAKE AWAY CHOICES

- The best choice (but still high in fat and not part of the WELL diet) is often fish and chips. But it has to be grilled fish and "NO ADDED SALT" – sometimes you have to get in quick to ask for that!!!!

- Another better (but not good) option may be barbequed chicken and chips (without skin and without added salt).

- REMEMBER both these options still are not part of the WELL diet but are better choices if there is absolutely nothing else and if cooking yourself is not an option.

REDUCING FAT
ON THE WAT DIET

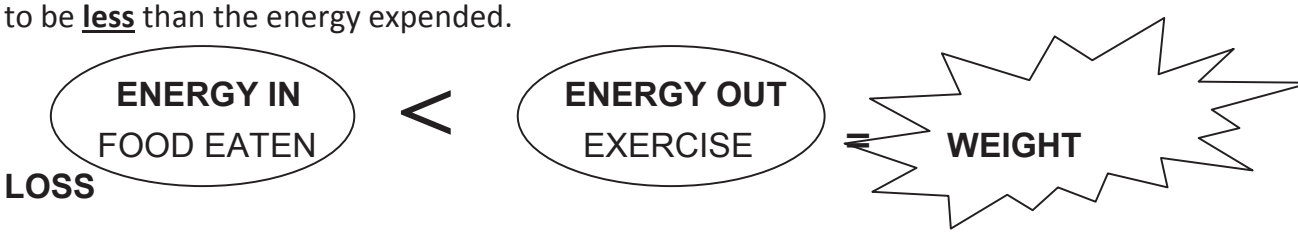
The WAT Diet involves a reduction in fat intake to result in significant loss of body fat.

Fat:
Fat is a very concentrated source of energy.
In fact, 1 gram of fat (from any source) will provide you with 37 kJ (or 9 calories), whereas 1 gram or protein or carbohydrate will provide 16-17 kJ (or 4 calories). So you can see that fat is much more **energy dense** than other nutrients.

How much fat do we need?

The human body requires only a small amount of fat to provide us with fat-soluble vitamins and essential fatty acids. However, most Australians eat a lot more than they need. Excess fat (of any kind) will be stored in the body as fat.

Weight Loss:
For weight loss (fat loss) to occur, the energy consumed (from all foods) needs to be **less** than the energy expended.



Different types of fat:
Fats are categorised as saturated, mono-unsaturated or poly-unsaturated. Most foods contain a mixture of these different types of fats.

- ***Saturated fats*** occur in both animal and vegetable foods (a popular myth is that saturated fat comes only from animal products). Saturated fat is found in fatty meats, full fat dairy products, palm oil, coconut oil, chocolate, baked foods etc. Saturated fats should be limited as they increase LDL cholesterol and are therefore a risk factor for heart disease.
- ***Mono-unsaturated fats*** are found in olive, peanut and canola oils, avocados, peanuts, eggs, chicken, some fish, macadamia and hazelnuts. Mono-unsaturated fats actually increase the protective HDL cholesterol.
- ***Poly-unsaturated fats*** are found in vegetable oils, margarines, fish, nuts and seeds.

Just remember that all types of fat provide the same amount of energy (kilojoules) and can all cause weight gain.

Fat sources:
Some common sources of fat include fried foods, pies, pasties, sausage rolls, chocolates, cakes, biscuits, pastries, fatty meats (sausages, salami etc), ice cream, cream, cheese, full fat dairy products, full fat mayonnaise and salad dressings, salted chips and nuts, pizza, oil, butter, margarine and takeaway food.

Fat on the WAT Diet:

The WAT Diet is a low fat diet. We want you to reduce the amount of fat you consume. So depending on how much you consume now, we need to you to significantly decrease this.

HINTS FOR DECREASING FAT INTAKE

- Cut out spreads such as margarine as much as possible, or choose a reduced fat version and spread very thinly.
- Use low fat or skim dairy products. Skim milk has up to 0.1% fat, whereas low and reduced fat versions have up to 2% fat. Full fat milk has about 4% fat.
- Cheese is very high in fat (~35%). Try lower fat versions like low fat cottage and ricotta.
- Try low fat ice creams and custards instead of full fat versions.
- Avoid take away foods.
- Use lean meat, trim off all visible fat before cooking and remove the skin off chicken.
- Choose canned tuna in spring water rather than oil or brine.
- Use lower fat cooking methods such as grilling, steaming or microwaving and always avoid frying foods.
- Don't add fat for flavour, be creative with herbs, spices, pepper, garlic, ginger, wine etc.
- Make your own fat free dressings.
- If you have to add fat, use a very light spray of oil or use a pastry brush to brush a small amount onto the food instead of pouring oil straight from the bottle.
- Be careful with "light", "lower fat", and "fat reduced" and "no added fat" claims on food labels. Often these foods still contain too much fat (eg light olive oil is just light in colour!)
- A "low cholesterol" or "no cholesterol" claim does NOT mean low/no fat. All vegetable products are cholesterol free (it is only found in animal products), but they may still contain fat (e.g. all olive oil varieties are cholesterol free but are 100% mono-unsaturated fat). To reduce your cholesterol you need to eat less fat, especially saturated fat, and maintain a healthy weight.

Average fat content of certain foods:

Food Product	**Fat (g) per 100g**	**Fat (g) per serve**
Oil, all types	100	1 tsp has 4.5g fat
Margarine regular	80.4	1 tsp has 3.9g fat
Peanuts, salted/unsalted	47.3	¼ cup has 18g fat
Salami	37.6	1 slice (23g) has 8.6g fat
Cream, thickened	36.8	1 tblsp has 7.4g fat
Cheese, cheddar/parmesan	33.8	1 slice (20g) has 6.7g fat
Potato chips, flavoured	33.4	1 50g pkt has 16.7g fat
Mayonnaise, regular fat	32.3	1 tblsp has 6.4g fat
Milk chocolate	27.4	1 regular Mars Bar has 11g fat
Cake, chocolate mud cake	24.6	1 slice has 17.2g fat
Chocolate chip biscuit	24	1 biscuit has 3g fat
Salad dressing	23.8	1 tblsp has 4.8g fat
Sausage Roll	23.6	1 sausage roll has 33g fat
Tuna, canned in oil	23.2	1 small can (95g) has 2g fat
Danish pastry	19.6	1 piece has 27g fat
Sausage, beef, grilled	18.2	1 thin snag (44g) has 8.1g fat
Bacon, middle, grilled	14.6	1 rasher (27g) 4g fat
Mayonnaise, low fat	13.1	1 tblsp has 2.8g fat
Meat Pie	11.8	1 pie has 20.5g fat
Ice cream, regular vanilla	10.6	1 cup has 15g fat
Steak, fillet, grilled	7.6	1 medium steak (65g) has 5g fat
Milk, full cream	3.8	1 cup has 9.8g fat
Rice cakes	3.4	2 cakes has 0.7g fat
Bread, grain	2.9	1 sandwich slice has 1.0g fat
Cheese, Low Fat Cottage	1.2	1 tblsp has 0.22g fat
Baked beans	0.6	1 cup has 1.6g fat
Salad dressing, light	0.1	1 tblsp has 0.02g fat
Milk, skim	0.1	1 cup has 0.3g fat
Banana	0.1	1 banana has 0.1g fat
Potato, boiled	0.1	1 potato has 0.1g fat

ALCOHOL
ON THE WAT DIET

In any weight loss diet, alcohol intake must be moderated. On the WAT diet we are asking you to limit your intake of alcohol to a maximum of **4 standard drinks a week**.

Alcohol often contributes to excess weight for two reasons:

- It is high in energy (kilojoules/calories).
    - Alcohol provides 29kJ per gram which is nearly double that provided by protein and carbohydrate (16-17kJ/g) but not quite as much per gram as fat (37kJ/g)

- It is often consumed with other high-energy foods such as chips, nuts and soft drink.

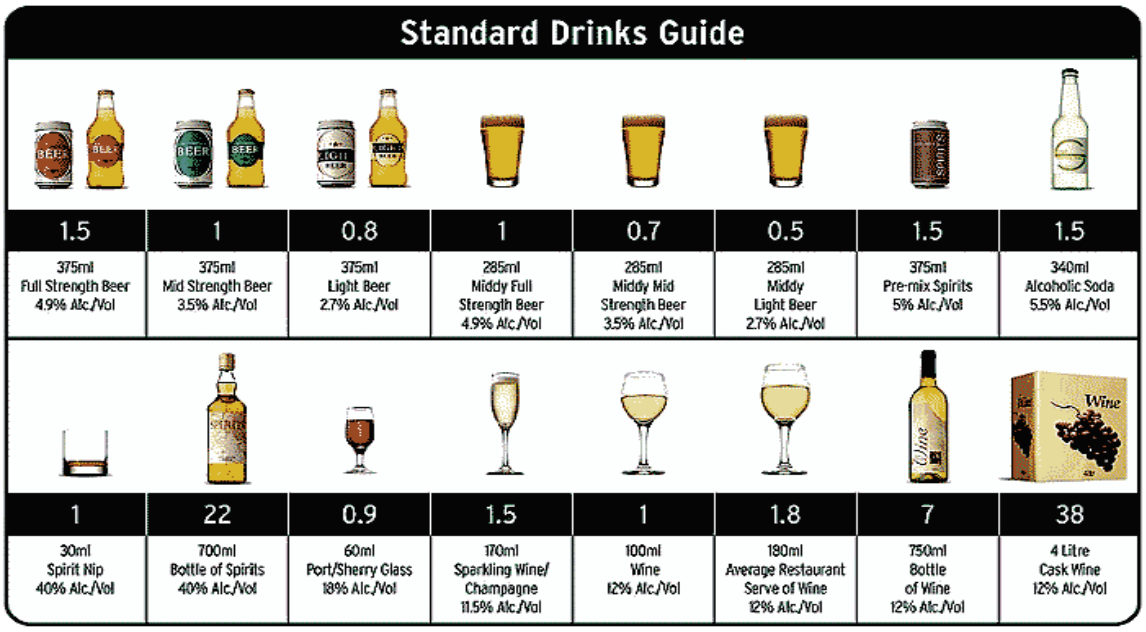
Alcohol can still be enjoyed as part of a weight loss diet but IN MODERATION! So depending on how much alcohol you drink, you may need to cut down a little bit or quite a lot!!

WHAT IS A STANDARD DRINK?

One standard alcoholic drink contains about 14g alcohol.
A common mistake is to think that one drink is the same as one "standard drink". In fact the difference can be quite significant. One standard drink of wine is 100ml but most wine glasses hold at least 200ml. There are seven standard drinks in a regular bottle of wine.

So remember:

One 200ml glass of wine is actually 2 standard drinks
One can or stubby of heavy beer is 1.5 standard drinks
One 375ml can of pre mixed spirits is 1.5 standard drinks



HINTS FOR DECREASING ALCOHOL INTAKE

- Space out your drinks.
- Alternate with water / plain mineral water / diet soft drink.
- Put your drink down between sips.
- Drink from a smaller glass.
- Dilute the alcohol you do drink so the drink lasts longer:
    - With soda water, tonic water or plain mineral water (e.g. wine or whisky)
    - Try mixing beer and diet lemonade
- Cut back on the amount of alcohol you use making the drink:
    - Use half nips of spirits
    - Drink light beer (less total kilojoules than heavy beer)
- If you are thirsty, drink something that will actually help re-hydrate you. When your body tells you that you're thirsty, you are already partially dehydrated. When this happens the best thing you can drink is water. That is what your body needs.
- Always order a jug of water when out for dinner so you can sip on it between alcoholic drinks.
- Use lower alcohol mixers:
    - Choose diet versions of your favourite mixers. You can get just about any mixer in a diet form - even diet ginger ale and diet tonic water!

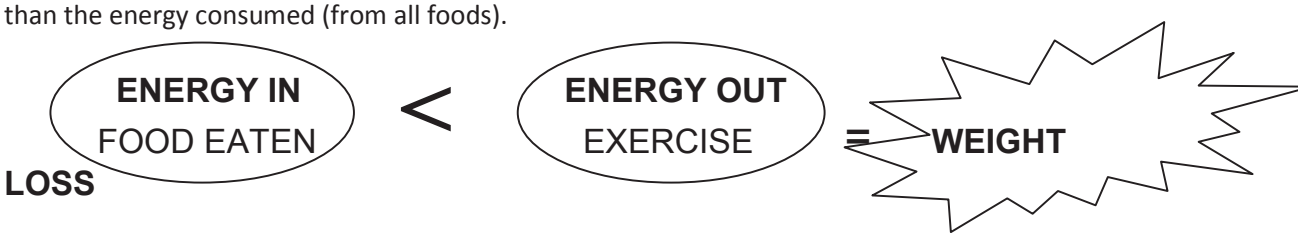
Average kilojoule (kJ) content of certain alcoholic drinks:

Drink	**Energy (kJ) per serve**
Beer, heavy	1 x 375 ml can has 550kJ (1.5 standard drinks)
Beer, light	1 x 375 ml can has 393kJ (0.8 standard drinks)
Wine, red or white	1 x 150 ml wine glass has 425kJ (1.5 standard drinks)
Wine, red or white	1 x 200 ml wine glass has 565kJ (2.0 standard drinks)
Wine, white sparkling	1 x 150 ml glass has 406 kJ
Rum	1 x 30ml nip has 255kJ (1.0 standard drink)
Mixed Drink,Rum&Cola	30ml rum & 200ml cola has 605kJ
Mixed Drink,Rum&DietCola	30ml rum & 200ml diet cola has 259kJ
Mixed Drink,Pre-Mixed,Canned,Rum&Cola	1 x 375 ml can has 984kJ (1.5 standard drinks)
Gin	1 x 30ml nip has 260kJ (1.0 standard drink)
Mixed Drink,Gin&Tonic	30ml gin & 200ml tonic has 547kJ
Mixed Drink,Gin&DietTonic	30ml gin & 200ml diet tonic has 256kJ
Mixed Drink,Pre-Mixed,Canned,Gin&Tonic	1 x 375 ml can has 903kJ (1.5 standard drinks)
Vodka	1 x 30ml nip has 256kJ (1.0 standard drink)
Mixed Drink,Vodka&Orange	30ml vodka & 200ml orange juice has 558kJ
Mixed Drink,Pre-Mixed,Canned,Vodka&Orange	1 x 375 ml can has 890kJ (1.5 standard drinks)
Mixed Drink,Pre-Mixed,Vodka,Lemon,Lime&Soda	1 x 375 ml can has 1938kJ (1.5 standard drinks)
Mixed Drink,Premixed,Canned,Brandy,Lime&Soda	1 x 375 ml can has 1857kJ (1.5 standard drinks)
Port	1 x 55ml glass has 341kJ
Liqueur,Cream-Based,Coffee Flavour	1 x 30ml nip has 436kJ

PHYSICAL ACTIVITY

Physical activity is a VITAL part of <u>any</u> weight loss program.

Weight Loss:
For weight loss (fat loss) to occur, the energy expended (physical activity) needs to be **<u>greater</u>** than the energy consumed (from all foods).



Physical Activity Goal on the Weight loss program: 30 minutes of MODERATE intensity AEROBIC physical activity on all or most days of the week

<u>What does **aerobic** physical activity mean?</u>
Aerobic exercise is continuous in nature, involves large muscle groups and increases your heart rate. Examples include, brisk walking, jogging, stair climbing, cycling, swimming, cross-country skiing, rowing, aerobics and dancing. Other sports like tennis and basketball can also incorporate an aerobic component, but due to continual stoping and starting you will need to increase your total time i.e. longer than 30 minutes.

<u>What does **moderate** intensity mean?</u>
Moderate intensity means the exercise should feel "somewhat hard" causing an increase in heart rate. A leisurely walk would not achieve this. A fast/brisk power walk with large steps and large arm movements would cause an increase in heart rate and thus burn more energy. Incorporating a hill or a light jog is another way of increasing your heart rate. Remember, as you get fitter, your body becomes more efficient as it adapts to the change. Because of this, you need to constantly increase the difficulty of the exercise in order to elevate your heart rate and continue weight loss.

<u>How do you **determine** moderate intensity physical activity? (Am I exercising hard enough?)</u>
Method 1
The Borg **Rate of Perceived Exertion** scale is one method to gauge the intensity of your physical activity. This scale assigns a number for different levels of exercise intensity. Moderate intensity would be classed as somewhat hard at 12-13.

very, very light			very light			fairly light			**somewhat hard**			hard			very hard			very, very hard		
6	7	8	9	10	11	**12**		**13**	14		15	16		17	18		19		20	

Method 2

Your heart rate is a measure of how hard you are physically exerting yourself. So one way to determine moderate intensity exercise is to calculate a **Target Heart Rate** to aim for while your exercising. The way to do this is to work out a range between 60-79% of your maximum heart rate.

Taking your heart rate: The pulse is most commonly taken either at the carotid artery on the neck or at the wrist. Use your index and middle fingers to locate your pulse – never use your thumb; it has a pulse of its own. Use a very light touch and avoid pressing hard. Find your pulse and count the number of beats in 60 seconds, the first beat you feel is counted as zero.

To work out a target heart rate:

1. Work out your Maximum Heart Rate= **220 - your age = a**
2. Determine 60-79% of this figure. **(a X 0.6) to (a X 0.79)**

Example

Bill is 56 years old. Therefore his maximum heart rate is 220 minus 56.

220 – 56 = **164** beats per minute (bpm)

To find 60% of 164, multiply by 0.6 164 X 0.6 = **98** bpm

To find 79% of 164, multiply by 0.79 164 X 0.79 = **130** bpm

Bill's target heart rate for moderate intensity is: **98-130** bpm

Class of intensity	Method 1 Rate of perceived exertion	Method 2 % Max Heart Rate
Very light	< 10	< 35%
Light	10 – 11	35 - 59%
Somewhat hard **(Moderate)**	**12 – 13**	**60 - 79%**
Heavy	14 – 16	80 - 89%
Very heavy	> 16	> 90%

Note for people on Blood Pressure Medication***

- If you are currently taking diuretics, make sure you keep well hydrated, as you are more at risk of becoming dehydrated during exercise so drink plenty of water!
- If you are taking beta-blockers your heart rate may not increase with exercise due to the effect of the medication. In this case, use the Rate of Perceived Exertion scale rather than Target Heart Rate.

General safety tips

- ✓ Keep breathing! Never hold your breath while exercising.
- ✓ Always warm up by gradually increasing the intensity (walk before running) and cool down (slow down back to a walk) for a minimum of 5 – 10 minutes at the start and end of every exercise session.
- ✓ Always stretch the appropriate muscles in use after the warm up and cool down (approx 30sec). Never stretch cold muscles!
- ✓ Always take a water bottle and keep well hydrated.
- ✓ Dress appropriately to avoid over heating and wear appropriate footwear (how old are your sneakers????). Old sneakers increase the amount of force going through your joints and thus increase risk of injury.
- ✓ Wear appropriate protective gear i.e. Bike helmets.
- ✓ Always gradually increase the intensity over time.
- ✓ Stop if experiencing pain.
- ✓ Ring or email Lisa (lpne@deakin.edu.au) with any queries, concerns or questions!
- ✓ IF IN DOUBT ASK FIRST.

For a CHALLENGE

- ➢ For a change, walk with a friend who is fitter or walks faster than you (and keep up!).
- ➢ Try carrying hand weights and make large arm swinging actions while walking.
- ➢ Try underwater running rather than swimming.
- ➢ Add a few hills in your walk.
- ➢ Introduce a light jog intermittently in your walk eg. a 30 sec jog every 5 minutes.
- ➢ Try a new activity!

Appendix J

Information given to subjects following the portion control diet in study three

The WAT Study

This diet is designed to REDUCE the portion sizes of the meals you consume. You do not have to change what you are eating, just the AMOUNT of what you are eating.

Large portion sizes are a cause for weight gain and by following the below tips, weight loss should occur.

Here are some guidelines to follow:

1. **Use the provided plate**
   This plate is probably smaller than your own. This makes a little food look like a lot, and research suggests that eating on smaller plates helps people eat less.

2. **Eat mindfully**
   Take time to enjoy the taste and texture of food – and the experience of feeling full.

3. **Avoid distractions**
   You're more likely to notice how much you eat if you don't multitask. So avoid eating while driving, reading or watching TV.

4. **Follow the 80 percent rule**
   Stop eating before you feel completely full. This guideline fits in with eating mindfully (dot point 2)

These tips may be easy to follow at home, but what happens when you are eating out at a restaurant?

1. **Just get an appetizer or two**
   Or share the entrée

2. **Doggy bag it**
   Bring half of your meal home to enjoy on another day. Get the extra food wrapped up early so there's less temptation.

3. **Avoid "value" meals**
   You may get more food but the extra calories, saturated fat, and sodium will probably hurt your heart and waist more than help your wallet.

4. **Order water**
   The average caloric intake has increased in Australians over the past 20 years, and about half of the increase comes from sugar sweetened drinks, which are often packed with calories and are easily consumed.

Appendix K

CHAMPS questionnaire

CHAMPS Activities Questionnaire

Developed by
Institute for Health and Aging
University of California San Francisco (UCSF)

Stanford Center for Research in Disease Prevention
Stanford University

Instructions: We are interested in finding out about the kinds of activities you do as part of your everyday life. You will be asked about activities you do at work, to get from place to place, as part of your house and yard work, and in your spare time for recreation, exercise or sport.

1. Which of the following statements best describes how active you have been during the past **2** weeks, that is, had hobbies, work, social activities, or other activities that kept you busy? *(Tick one box)*

Not at all active	☐ 1
A little active	☐ 2
Fairly active	☐ 3
Quite active	☐ 4
Very active	☐ 5
Extremely active	☐ 6

2. Which of the following statements best describes how physically active you have been during the past 2 weeks, that is, done activities such as brisk walking, swimming, dancing, general conditioning, or recreational sports? *(Tick one box)*

Not at all active	☐ 1
A little active	☐ 2
Fairly active	☐ 3
Quite active	☐ 4
Very active	☐ 5
Extremely active	☐ 6

3. During the past 2 weeks, about how many flights of stairs did you climb during a typical day? (one flight = 12-15 steps, equivalent to going from one floor to another) *(Tick one box)*

None	☐ 1
Less than one	☐ 2
1-2 flights	☐ 3
3-4 flights	☐ 4
5 or more flights	☐ 5

Instructions: Read Carefully

4. Think about the past 4 weeks. The next few pages list various activities you might have done. Before you begin, please review the following steps and examples:

Step #1: Number of times each week

- For each activity, write on the line provided how many times each week, on average, you did that activity.
- If you did an activity less than once a week or not at all, please write **"0"** on the line provided

  For example, if you did not do the activity at all or did it less than once a week during the past 4 weeks **(see Example A)**

Example A **Step #1**

Activities	**Number of times a week** (If none, write "0")	Less than 1 hr/wk	1-2½ hrs/wk	3-4½ hrs/wk	5-6½ hrs/wk	7-8½ hrs/wk	9 or more hrs/wk
Mow lawns	Times a week **0** →	A	B	C	D	E	F

Step #2: Total time, on average, each week

- If you did the activity at least once a week, circle one letter representing how much total time, on average, you spent doing it each week ***(see Example B)***

  For example, if you did the activity on average 3 times a week for a total of 1½ hours:

Example B **Step #1** ← **Step #2** →

Activities	**Number of times a week** (If none, write "0")	Less than 1 hr/wk	1-2½ hrs/wk	3-4½ hrs/wk	5-6½ hrs/wk	7-8½ hrs/wk	9 or more hrs/wk
Use computer	Times a week **3** →	A	(B)	C	D	E	F

Think about the past 2 weeks. For each activity, please write **HOW MANY TIMES** each week, on average you did it. Next, please circle one letter representing how much **TOTAL TIME**, on average, you spent doing that activity each week.

Social Activities:	**Number of times a week** (If none, write "0")	Less than 1 hr a week 1	1-2½ hrs a week 2	3-4½ hrs a week 3	5-6½ hrs a week 4	7-8½ hrs a week 5	9 or more hrs a week 6
a. Visit with friend or family (other than those you live with)	Times a week _____ ➔	A	B	C	D	E	F
b. Go out for a meal or drink with friends	Times a week _____ ➔	A	B	C	D	E	F
c. Do volunteer work	Times a week _____ ➔	A	B	C	D	E	F
d. Attend church or take part in church activities	Times a week _____ ➔	A	B	C	D	E	F
e. Attend other club or group meetings	Times a week _____ ➔	A	B	C	D	E	F
e. Talk on the phone (when not at work)	Times a week _____ ➔	A	B	C	D	E	F

Recreation and Hobbies:	**Number of times a week** (If none, write "0")	Less than 1 hr a week 1	1-2½ hrs a week 2	3-4½ hrs a week 3	5-6½ hrs a week 4	7-8½ hrs a week 5	9 or more hrs a week 6
g. Use a computer (<u>not</u> work related)……	Times a week _____ ➔	A	B	C	D	E	F
h. Dance (such as line, ballroom) (do not count aerobic dance here) …….	Times a week _____ ➔	A	B	C	D	E	F
i. Do woodwork, painting, drawing, or other arts or crafts ……	Times a week _____ ➔	A	B	C	D	E	F
j. Play golf, riding a cart (count walking time only) …….	Times a week _____ ➔	A	B	C	D	E	F
k. Play golf, carrying or pulling your equipment (count walking time only)	Times a week _____ ➔	A	B	C	D	E	F
l. Attend a concert, movie, lecture, or sporting event …….	Times a week _____ ➔	A	B	C	D	E	F
m. Play cards, or board games with other people …….	Times a week _____ ➔	A	B	C	D	E	F

Think about the past 2 weeks. For each activity, please write **HOW MANY TIMES** each week, on average you did it. Next, please circle one letter representing how much **TOTAL TIME**, on average, you spent doing that activity each week.

Recreation and Hobbies:	**Number of times a week** (If none, write "0")	Less than 1 hr a week 1	1-2½ hrs a week 2	3-4½ hrs a week 3	5-6½ hrs a week 4	7-8½ hrs a week 5	9 or more hrs a week 6
n. Shoot pool or billiards ……	Times a week _____ ➔	A	B	C	D	E	F
o. Play tennis …….	Times a week _____ ➔	A	B	C	D	E	F
p. Play a musical instrument ……	Times a week _____ ➔	A	B	C	D	E	F
q. Read ……	Times a week _____ ➔	A	B	C	D	E	F

Work Around the House:	**Number of times a week** (If none, write "0")	Less than 1 hr a week 1	1-2½ hrs a week 2	3-4½ hrs a week 3	5-6½ hrs a week 4	7-8½ hrs a week 5	9 or more hrs a week 6
r. Do heavy work around the house (such as washing windows, cleaning gutters)..	Times a week _____ ➔	A	B	C	D	E	F
s. Do light work around the house (such as sweeping or vacuuming)…….	Times a week _____ ➔	A	B	C	D	E	F

Work Around the House:	**Number of times a week** (If none, write "0")	Less than 1 hr a week 1	1-2½ hrs a week 2	3-4½ hrs a week 3	5-6½ hrs a week 4	7-8½ hrs a week 5	9 or more hrs a week 6
t. Do heavy gardening (such as digging in garden, raking)…….	Times a week _____ ➔	A	B	C	D	E	F
u. Do light gardening (such as watering plants) ……	Times a week _____ ➔	A	B	C	D	E	F
v. Work on your car, truck, lawn mower, or other machinery …..	Times a week _____ ➔	A	B	C	D	E	F

Walking and Jogging:	**Number of times a week** (If none, write "0")	Less than 1 hr a week 1	1-2½ hrs a week 2	3-4½ hrs a week 3	5-6½ hrs a week 4	7-8½ hrs a week 5	9 or more hrs a week 6
w. Walk uphill or hike uphill (count only uphill part) ……..	Times a week _____ ➔	A	B	C	D	E	F
x. Walk leisurely for exercise or pleasure ……	Times a week _____ ➔	A	B	C	D	E	F
z. Walk to do errands (such as to/from a shop) (count walk time only)	Times a week _____ ➔	A	B	C	D	E	F

Walking and Jogging:	**Number of times a week** (If none, write "0")	Less than 1 hr a week 1	1-2½ hrs a week 2	3-4½ hrs a week 3	5-6½ hrs a week 4	7-8½ hrs a week 5	9 or more hrs a week 6
aa. Walk fast or briskly for exercise (do not count walking leisurely or uphill)	Times a week _____ ➔	A	B	C	D	E	F
bb. Jog or run	Times a week _____ ➔	A	B	C	D	E	F

Other Types of Exercise:	**Number of times a week** (If none, write "0")	Less than 1 hr a week 1	1-2½ hrs a week 2	3-4½ hrs a week 3	5-6½ hrs a week 4	7-8½ hrs a week 5	9 or more hrs a week 6
cc. Ride a bicycle or stationary cycle using legs only	Times a week _____ ➔	A	B	C	D	E	F
dd. Do aerobic machines involving arms and legs (such as rowing or cross-country ski machines)	Times a week _____ ➔	A	B	C	D	E	F
ee. Do stair or step machine	Times a week _____ ➔	A	B	C	D	E	F

Other Types of Exercise:	**Number of times a week** (If none, write "0")	Less than 1 hr a week 1	1-2½ hrs a week 2	3-4½ hrs a week 3	5-6½ hrs a week 4	7-8½ hrs a week 5	9 or more hrs a week 6
ff. Swim gently	Times a week _____ ➔	A	B	C	D	E	F
gg. Swim moderately or fast	Times a week _____ ➔	A	B	C	D	E	F
hh. Do water exercises (do not count swimming	Times a week _____ ➔	A	B	C	D	E	F
ii. Do stretching or flexibility exercises (do not count yoga or Tai-chi)	Times a week _____ ➔	A	B	C	D	E	F
jj. Do yoga or Tai-chi	Times a week _____ ➔	A	B	C	D	E	F
kk. Do aerobics or aerobic dancing	Times a week _____ ➔	A	B	C	D	E	F
ll. Do moderate to heavy strength training (such as hand held weights or more than 5 lbs, weight machines, or push ups	Times a week _____ ➔	A	B	C	D	E	F

Think about the past 2 weeks. For each activity, please write **HOW MANY TIMES** each week, on average you did it. Next, please circle one letter representing how much **TOTAL TIME**, on average, you spent doing that activity each week.

Other Types of Exercise:	**Number of times a week** (If none, write "0")	Less than 1 hr a week 1	1-2½ hrs a week 2	3-4½ hrs a week 3	5-6½ hrs a week 4	7-8½ hrs a week 5	9 or more hrs a week 6
mm. Do light strength training (such as hand held weights of 5lbs or less or elastic bands	Times a week _____ ➔	A	B	C	D	E	F
nn. Do light calisthenics or chair exercises (do not count strength training)	Times a week _____ ➔	A	B	C	D	E	F
oo. Play basketball, football, soccer, or racquetball/squash (do not count time on sidelines)	Times a week _____ ➔	A	B	C	D	E	F
pp. Do other types of physical activity not previously mentioned (please specify) _____	Times a week _____ ➔	A	B	C	D	E	F

Thank You For Your Time

Appendix L

Recruitment flyer for study two

ARE YOU INTERESTED IN A FREE BREAKFAST AND DINNER?

WE NEED VOLUNTEERS!

Deakin University **is looking for volunteers who would like to participate in a study investigating fat consumption and satiety (feeling of fullness)**

Are you:

- Over 18 years of age?
- A non- smoker?
- Willing to consume 3 different types of breakfasts on separate days?
- Willing to taste test milk and custard?

CONTACT: Lisa Newman (03) 9251 7286 or email sensory@deakin.edu.au for more details.

Appendix M

Recruitment flyer for study three

DEAKIN UNIVERSITY AUSTRALIA

ARE YOU KEEN TO LOSE WEIGHT?

WE NEED VOLUNTEERS!

Deakin University **is looking for volunteers who are keen to LOSE WEIGHT to participate in a research study.**

Subjects will receive a **$50 Coles Myer voucher**

Are you:

- 18 – 75 years?
- A non- smoker?
- Willing to consume a modified diet and engage in physical activity for a period of 6 weeks?
- Willing to taste test milk, custard, cream cheese, yoghurt and mousse?
- Willing to attend Deakin University, Burwood Campus for two appointments?

CONTACT: Lisa Newman (03) 9251 7286 or email sensory@deakin.edu.au for more details.