The Influence of Diet and Genes in Fat Taste

by

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Abstract

The worldwide prevalence of overweight and obesity is rising and has become a serious public health issue in developed nations. Although obesity is multifactorial and complex, one of the key contributors to the development of obesity is excess energy intake, particularly from high-fat, energy-dense foods. The ability to detect fatty acid in the oral cavity, known as fat taste, may be a factor that is driving excess fat consumption. Five fat taste receptors have recently been identified in humans (CD36, FFAR2, FFAR4, GPR84 and KCNA2) that are responsible for fat taste perception, each having different specificities to fatty acid chain lengths and levels of saturation. Fat taste is initiated by the chemoreception of fatty acid by fat taste receptors on taste bud cells, housed within structures known as papillae located in the oral cavity. Taste papillae are located at regional clusters on the tongue: fungiform (anterior tongue), foliate (posterior lateral tongue) and circumvallate (posterior medial tongue). Fat taste has a role in the regulation of energy intake, where a number of satiety signals are stimulated following activation of fat taste receptors by fatty acid. Individuals with impaired fat taste have a reduced satiety response following ingestion and digestion of fatty foods, and therefore causes passive overconsumption. However, sensitivity to fat taste can be modulated by altering habitual dietary fat intake, with low-fat intake increasing sensitivity to fat taste and high-fat intake decreasing sensitivity. The likely mechanism for this is downregulation of fat taste receptors following oral fat exposure and upregulation when oral fat is absent, although this has only been demonstrated in animal models.

Fat taste sensitivity is measured in humans as fat taste threshold (FTT), which is defined as the lowest amount of fatty acid required to elicit a detectable taste response.
Higher thresholds imply lower sensitivity, and vice versa. There is a large variation in FTT amongst individuals, with some studies showing a 40-fold range in FTT concentration within a sample of participants. There is some evidence to suggest there is an association between FTT and obesity, although this point is contentious and requires further research. The aim of this thesis is to assess whether individual variation in FTT influences obesity and dietary intake (both habitual and acute intake). Further, it aims to investigate the factors that influence fat taste sensitivity and fat taste receptor gene expression, specifically the role that environment and genetics have on modulating sensitivity. A secondary aim was to assess the regional sensitivity of fat taste between taste papillae clusters. Four studies were conducted as part of this thesis to assess these aims, and findings from each study are summarised below.

**Study 1** assessed the associations between FTT, obesity, fat intake, and liking of fatty foods. FTT was assessed in 69 Australian females (34 twin pairs, 1 individual; mean age 41.3 (15.6) (SD) years; mean body mass index 26.3 (5.7) kg/m2), by a 3-alternate forced choice (3-AFC) methodology and transformed to an ordinal scale (FTT rank) ranging from 0-12. Food liking was assessed by hedonic ratings of seven high-fat and reduced-fat foods; and a 24-hour food recall and food frequency questionnaire were completed to assess acute and habitual dietary intake, respectively. Linear mixed regression models were fitted with associations adjusted for twin pair to prevent the clustering effect of twins, and energy intake where appropriate. The results showed that participants who had a higher FTT rank (were less sensitive to fat taste) consumed significantly more energy from fat ($\beta = 0.11$ [95% CI: 0.01, 0.22]), and conversely consumed significantly less energy from carbohydrate ($\beta = -0.11 [-0.19, -0.03]$) according to the 24-hour food recall. While they did not reach significance, the associations between FTT rank and energy from saturated ($\hat{\beta} = 0.20$) and
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Monounsaturated fat (β = 0.22) were large and trended in the expected direction. Similarly, participants with higher FTT rank had greater daily habitual consumption of foods from high-fat dairy (β = 1.03 [0.12, 2.18]), grain & cereal (β = 0.77 [0.22, 1.32]), and meat & meat alternative (β = 0.66 [0.16, 1.15]) food groups according to a food frequency questionnaire (FFQ). The large association between FTT rank and consumption of high-fat dairy matches the macronutrient intake data from the 24-hour recall, as high-fat dairy is a major source of saturated and monounsaturated fat. Similarly, the association between FTT rank, meat & meat alternatives, and grain & cereal was likely due to fat in meat products and the fatty spreads that are commonly consumed with grain and cereal products, particularly white bread. No association was observed between FTT rank and anthropometric measurements or liking of fatty foods. Therefore, fat taste sensitivity appears to be associated with short-term and habitual fat intake, but not body size or hedonics in this group of females.

**Study 2** assessed the effect of 8-week low-fat or high-fat dietary intake on FTT whilst maintaining baseline body weight (< ±2.0kg). In addition, this study aimed to assess heritability of FTT, and explore the effect of genetics on diet-mediated changes to FTT. A co-twin randomised controlled trial including 44 pairs (mean age 43.7 (15.4) years; 34 monozygotic, 10 dizygotic; 33 female, 10 male, 1 gender-discordant) was conducted. Twins within a pair were randomly allocated to an 8-week low-fat (< 20% energy fat) or high-fat (> 35% energy fat) diet. FTT was assessed by a 3-AFC methodology (transformed to FTT rank) at baseline, 4 and 8 weeks. Linear mixed models were fitted to assess the effect of diet on FTT rank, and the diet effect modification due to zygosity. Associations were adjusted for twin pair to prevent the clustering effect of twins. A variance components model was fitted to calculate baseline heritability. Following 8 weeks, FTT rank decreased from 6.8 to 2.6 on the
low-fat diet (-4.2 [-5.4, -3.0], \(P < 0.001\)), and increased from 6.9 to 8.4 on the high-fat diet (+1.4 [0.2, 2.6], \(P = 0.017\)). There was a significant time-diet interaction for FTT rank \((P < 0.001)\). There was no evidence of zygosity effect modification on FTT rank (interaction time-diet-zygosity: \(P = 0.892\)) suggesting that genetics do not have a large role in diet-mediated changes to FTT. Furthermore, heritability of FTT rank was 8%. These results indicate that there is little to no genetic contribution on the regulation of FTT rank. Rather, environment, specifically dietary fat intake, is the main influencer of fat taste sensitivity, regardless of body weight.

**Study 3** assessed the effect of 8-week low-fat or high-fat dietary intake on fasting fat taste receptor gene expression (CD36, FFAR2, FFAR4, GPR84 and KCNA2) in fungiform papillae. A co-twin randomised controlled trial was conducted using a subset of the participants from **Study 2** (mean age 41.6 (17.0) years; 10 monozygotic, 3 dizygotic; 9 female pairs, 4 male pairs). Twins within a pair were randomly allocated an 8-week low-fat (< 20% energy fat) or high-fat (> 35% energy fat) diet. At baseline and week 8, fungiform papillae were biopsied and fat taste receptor gene expression was measured using real-time reverse transcription polymerase chain reaction (RT-PCR); and FTT was assessed by a 3-AFC methodology (transformed to FTT rank). Linear mixed models were fitted to assess the effect of diet on fat taste receptor gene expression. Associations were adjusted for twin pair to prevent the clustering effect of twins. Expression of FFAR4 increased from 0.84 to 1.16 on the low-fat diet (+0.32 [0.05, 0.58], \(P = 0.023\); time-diet interaction: \(P = 0.063\)). \(\Delta\) FFAR4 (\(\Delta\), week 8 – baseline) was associated with \(\Delta\) fat intake (g) (\(\hat{\beta} = -159.4, P < 0.001\)) and \(\Delta\) FTT rank (\(\hat{\beta} = -8.8, P = 0.016\)), indicating that as dietary fat intake decreases, FFAR4 is upregulated and fat taste becomes more sensitive. In addition, there was some evidence for a negative association between \(\Delta\) KCNA2 and \(\Delta\) polyunsaturated fat
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intake ($\beta = -72.7, P = 0.056$), suggesting that KCNA2 may have a role in regulating polyunsaturated fat intake. These results indicate that FFAR4 and KCNA2 may be responsible for regulating long-term satiety and desire to consume fatty foods. Conversely, CD36, GPR84 and FFAR2 are not related to changes in dietary fat intake in the fasted state.

Study 4 assessed the comparative regional sensitivities of papillae clusters to fatty acid stimulation. Twenty healthy weight participants, aged 19-42 years completed the study. A fatty acid (oleic acid, C18:1) or paraffin oil (control) were individually applied to six regions of the tongue (fungiform-left, fungiform-right, foliate-left, foliate-right, circumvallate-left, circumvallate-right), in a randomized order, in duplicate. Participants were asked to indicate presence of a taste response after each application. Foliate was the only region to significantly differ in proportion of positive taste responses between C18:1 and control ($P < 0.001$). Foliate also had a higher proportion of positive taste responses to C18:1 than fungiform ($P < 0.001$) or circumvallate ($P = 0.010$) regions. No differences were seen between the left and right side of the tongue for any papillae region ($P > 0.05$). Additionally, individuals more sensitive to fat taste were more likely to respond positively to C18:1 stimulation than less sensitive individuals in the foliate ($P = 0.007$) and circumvallate ($P = 0.058$) regions, but not the fungiform region ($P = 0.943$). The results from this study indicate that the foliate papillae region is the most sensitive region to stimulation by fatty acid.

The four studies conducted as part of this thesis advance the knowledge of fat taste function and its relation to nutrition and health. First, data from this thesis has reported that habitual dietary fat intake is the main influencer of fat taste sensitivity, with minimal effect of genetic factors. Low dietary fat intake increases sensitivity and high dietary fat intake attenuates sensitivity, regardless of weight status. The mechanism
for this changes appears to result from the regulation of fat taste receptor gene expression, particularly $FFAR4$ which is likely involved in the mediated long-term dietary fat intake. A greater expression of $FFAR4$ would trigger a greater satiety response following activation by fatty acid, leading to reduced intake of fatty food. $KCNA2$ may also support in regulating intake of polyunsaturated fat. Manipulating gene expression and receptor activation may be novel solutions for moderating satiety and reducing risk of overweight and obesity. Second, baseline associations between fat taste sensitivity and dietary fat intake suggest that less sensitive individuals are more likely to consume greater amounts of fat, particularly from monounsaturated and saturated fat sources. This association is likely due to variation in satiety response following fatty food consumption, rather than hedonics or desire to consume fatty food as there was no association observed between fat taste sensitivity and liking of fatty foods. Contrary to this, there was no direct association found between fat taste sensitivity and obesity. Obesity is multifactorial and complex, and it is likely that fat taste sensitivity is just one factor of many and may be more influential in some individuals than others. Third, perception of fatty acid was not equal between papillae clusters on the tongue, where foliate papillae clusters appeared to be the most sensitive region. Finally, the overall results from this thesis highlight the potential for manipulation of fat taste receptor expression and activation to stimulate increased satiety responses in individuals. This may be a novel pathway for appetite suppression, thereby reducing passive overconsumption of fatty foods and reducing risk of overweight and obesity.
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List of Abbreviations

3-AFC: 3-Alternate Forced Choice

BMI: Body Mass Index

C18:1: Oleic Acid

C18:2: Linoleic Acid

cDNA: Complimentary Deoxyribonucleic Acid

DZ: Dizygotic

FFA: Free Fatty Acid

FFAR: Free Fatty Acid Receptor

FFQ: Food Frequency Questionnaire

FTT: Fat Taste Threshold

FTT-F: Fat Taste Threshold-Fungiform Papillae

GIT: Gastrointestinal Tract

GPR: G Protein-Coupled Receptor

HF: High-Fat
List of Abbreviations

**LF**: Reduced-Fat

**MZ**: Monozygotic

**PUFA**: Polyunsaturated Fatty Acid

**RCT**: Randomised Controlled Trial

**RNA**: Ribonucleic Acid

**RT-PCR**: Reverse Transcription Polymerase Chain Reaction

**SD**: Standard Deviation

**TBC**: Taste Bud Cell

**TG**: Triglyceride

**TRA**: Twins Research Australia
Chapter One: Literature Review

An abridged version has been published in Flavour 2015, 4:5; doi.org/10.1186/2044-7248-4-5, ‘Is fat the sixth taste primary? Evidence and implications’.

1.1 Introduction

The sense of taste presumably evolved to inform us about the nutritious or toxic value of potential foods. The primary organ responsible for the sense of taste is the tongue, which contains the biological machinery (taste receptors) to identify non-volatile chemicals in foods and non-foods we place in our mouth. Once a food enters the mouth, the tongue aids in the manipulation of the food, assisting breakdown and bolus formation before swallowing the food. During this critical period of food manipulation, the tongue is sampling chemicals in the food, and when food chemicals activate taste receptors, signals are sent from the taste receptors to processing regions of the brain. The signals are decoded by the brain, and we perceive the taste of the food, which could be one of five distinct qualities: sweet, sour, salty, bitter and umami.

It is perhaps appropriate to classify taste as a nutrient-toxin detection system, with the qualities (sweet, etc.) informing us via an associated hedonic response of suitability to swallow or reject. For example, sweet elicited by sugars reflecting energy content, sour elicited by free hydrogen ions (H\(^+\)) reflecting excessive acid, umami elicited by glutamate and other amino acids reflecting protein content, salt elicited by sodium (Na\(^+\)) and other ions reflecting mineral content, and bitter reflecting potential toxins in foods. Excessive bitterness or sourness is aversive and informs that the food in our
mouth may cause harm and that the best action is to expectorate, whereas the qualities sweet, umami and salty are all appetitive within a relevant intensity range and inform that the food contains compounds we should ingest, in this case, essential nutrients such as sugars, proteins and minerals, respectively. As the taste system has evolved to detect the nutrients or toxins in foods prior to ingestion, it makes sense that fats, an essential energy-dense macronutrient required in limited amounts for energy and nutritional needs, would be detected through taste, as other macronutrients namely sugars and proteins are detected through the tastes of sweet and umami.

1.2 Fat Taste
Fat taste, also known as oleogustus (Running et al., 2015) and pinguis (Reed & Xia, 2015), is an area of increasing interest particularly in chemosensory and nutrition research with the possibility that it may be linked with dietary consumption of fatty foods. The intake and regulation of dietary fats is considered especially important in the development of overweight and obesity, given their high energy density and palatability alongside their ability to promote excess energy intake. The intake and regulation of fats in the obese state appears especially problematic given that obese persons have a higher preference for fatty foods compared with lean individuals, which represent significant portions of the obese diet.

Fat has been associated with texture, flavour release and thermal properties in foods (Fennema, 2007), but has only recently been accepted as a primary taste quality (Besnard et al., 2016; Keast & Costanzo, 2015; Running & Mattes, 2016). This may seem like an irrelevant academic point, but the taste system is only activated when a saliva-soluble component of a potential food activates receptors on taste cells. Adding
to the importance of the sense of taste is the interplay between taste cell activation and multiple digestive processes, therefore making the link between taste and fat intake very important, especially given the link dietary fat has with the development of obesity (Hooper et al., 2015).

For fat to be generally accepted as a taste, it must meet five criteria:

1. There must be a distinct class of affective stimuli. The stimuli responsible for fat taste are the breakdown products of triglycerides (TG) – fatty acids (Chale-Rush et al., 2007; Newman & Keast, 2013).

2. There should be transduction mechanisms including receptors to change the chemical code of the stimuli to electrical signal. Receptors for fat taste in humans include CD36, G protein-coupled receptor (GPR) 84, free fatty acid receptor (FFAR) 2 (also known as GPR43), FFAR4 (also known as GPR120), and delayed rectifying potassium channel KCNA2 (Liu et al., 2018).

3. There must be neurotransmission of the electrical signal to processing regions of the brain (De Araujo & Rolls, 2004; Rolls et al., 1999).

4. There should be perceptual independence from other taste qualities (Running et al., 2015).

5. Finally, there must be physiological effects after activation of taste bud cells (Mattes, 2001a; 2001b).

1.3 Fatty Acids as Stimuli

While it is well established that oxidised or reverted fatty acids or fatty acids at high concentrations are unpleasant to taste, the taste quality of fatty acids will vary according to their concentration in a food. The levels of fatty acids involved in fat taste
are low enough not to be considered unpleasant in unspoiled food, yet sufficient to activate fat taste receptors. For example, the concentrations of free fatty acids (FFA) required for detection are within ranges which may be inherently present in edible fresh and processed foods (0.1%–3% w/v) (Che Man et al., 1999; Koriyama et al., 2002), with potential for additional FFA made available through enzymatic hydrolysis by lingual lipase.

1.3.1 Lingual Lipase

Lipase enzymes are very important as they breakdown TG so that FFA can be transduced by cellular pathways. However, the presence and activity of lingual lipase in human saliva remains controversial. Data has suggested that lipolytic activity may be present in humans (Kulkarni & Mattes, 2014; Stewart et al., 2010), although it is unknown whether sufficient concentrations of lingual lipase are produced to liberate FFA from TAG in quantities large enough for taste detection, and whether this activity originates from endogenous sources (saliva) or oral microbes. The presence of lipase does appear to have an influence on fat taste with research showing that the addition of orlistat (lipase inhibitor) during testing increased TG thresholds indicating that the inability to hydrolyse TG in the oral cavity led to decreased fat taste sensitivity (Pepino et al., 2012). Regardless, the weight of evidence suggests that FFA in fatty foods is in sufficient concentrations to activate putative receptors on taste cells.
1.4 Fat Taste Receptors and Transduction

1.4.1 Gustatory Papillae

Fat taste is initiated by the chemoreception of fatty acids by fat taste receptors on taste bud cells (TBC) housed within structures known as papillae in the oral cavity. Papillae are located at regional clusters on the tongue: fungiform (anterior tongue), foliate (posterior lateral tongue), circumvallate (posterior medial tongue); and non-taste papillae: filiform (medial tongue) (Figure 1.1).

Figure 1.1 – Diagram of human tongue indicating the regional clusters of papillae.
1.4.2 CD36 Transporter

One of the mechanisms of oral fatty acid nutrient detection is via CD36, a fatty acid transporter (Abumrad, 2005). CD36 is found in the oral cavity on human TBCs, specifically within fungiform, circumvallate and foliate papillae (Liu et al., 2018; Simons et al., 2011). Relative ratios of CD36 within different papillae TBCs has not been investigated in humans, although there is a lower expression of CD36 within fungiform TBCs in rodents (Gilbertson et al., 2005). Genetic variants of CD36 have been associated with variation in oleic acid (C18:1) detection (Pepino et al., 2012), providing further evidence for a role of CD36 for fat taste in humans. Expression of CD36 on TBCs exhibits a short-term diurnal rhythm in rodents, where CD36 protein levels decrease promptly following feeding but return to pre-prandial levels after fasting (Martin et al., 2011).

1.4.3 G Protein-Coupled Receptors

It has been proposed that CD36 may work together with other possible receptors like GPRs in a signalling cascade to detect fatty acid (Galindo et al., 2012). FFAR4 is activated by long-chain fatty acids (LCFA) and medium-chain fatty acids (MCFA) initiating a peripheral signalling cascade that includes a release of calcium that activates the cation channel transient receptor potential channel type M5 (Liu et al., 2011). FFAR4 is expressed in the apical portion of types I and II TBCs in animal papillae (Cartoni et al., 2010; Matsumura et al., 2007; Sawamura et al., 2015) and, more recently, human circumvallate and fungiform papillae TBCs (Galindo et al., 2012; Liu et al., 2018), but not foliate papillae (Galindo et al., 2012). FFAR4 expression does not appear to have a strong diurnal rhythm like CD36, and is resistant to changes in expression levels after short-term dietary fat intake (Martin et al., 2011).
However, the effect of long-term or habitual dietary fat intake on FFAR4 expression is not known.

Other GPRs may also have a similar role in stimulating a signalling cascade in response to fatty acids. FFAR2 and GPR84, which are exclusively activated by short-chain fatty acids (SCFA) and MCFA, respectively, are expressed in human fungiform papillae (Liu et al., 2018). However, there is evidence to suggest that shorter fatty acid chain lengths elicit a taste quality independent to fat taste, suggested to be more similar to sour taste (Running et al., 2015). These receptors are greatly expressed in the gastrointestinal tract (GIT), and likely have a more important role in nutrient-sensing of SCFA produced from dietary fibre digestion by GIT biota (Kles & Chang, 2006).

FFAR1 has also been proposed as a candidate for fat taste function in rodent models (Cartoni et al., 2010), although the receptor is not present in human fungiform TBCs (Liu et al., 2018).

1.4.4 Delayed Rectifying Potassium Channels

Delayed rectifying potassium channels are known to be implicated in the transduction pathway of a variety of taste stimuli. Polyunsaturated fatty acids (PUFA) slow down delayed rectifying potassium channel polarisation on the foliate and circumvallate papillae taste cells which allow PUFA to be detected (Gilbertson, 1998). The delayed rectifying potassium channel KCNA2 was recently shown to be expressed in human fungiform papillae TBCs (Liu et al., 2018).
1.4.5 Neurotransmitter Release

A transduction mechanism that converts the chemical signal to an electrical signal is required to establish the taste component in dietary fat consumption. Previous studies suggested that the general chemoreception pathway starts from the fatty acids activating the receptor or ion channel and results in the complex cascade that leads to the cell depolarization. The neurotransmitters such as noradrenaline and serotonin will then be secreted towards afferent nerve fibres which trigger the orosensory perception (Dando & Roper, 2009). In general, neurotransmission of sensory stimulation is mediated through the thalamus. From there, the signal is relayed to the primary gustatory cortex which is responsible for detection and perception of the sensory characteristics of food, with periphery projections to the orbitofrontal cortex (Iannilli et al., 2012). These cortices contain neuron populations specific to individual taste sensations, with some evidence of neurons specific to fat texture (Rolls et al., 1999), viscosity and taste (Rolls et al., 2003; Verhagen et al., 2004). Further research is required relating specifically to neurotransmission of fat taste.

1.5 Perceptual Independence

For all tastants, perception of the taste runs along a sensory concentration continuum (Figure 1.2). At very low concentrations, fatty acid may be detected, albeit with no taste quality attached, i.e. the concentration is too low to be recognised (Keast & Roper, 2007). As the concentration increases, e.g. as a result of fat hydrolysis or spoiling within a food, fatty acid may then be tasted or recognised. Once the concentration of fatty acid is high enough for recognition and supra-threshold, the flavour is generally unpleasant. At the supra-threshold level, it is likely that sensory
systems other than taste are involved, for example smell or chemesthesis, therefore fat
taste quality is not equivalent to easily identified qualities such as sweet or salty. One
taste dimension for fatty acid that is reliably measurable is detection threshold, and
research has shown that this measure is independent of detection thresholds for other
basic tastes, thereby meeting the criteria for perceptual independence (Newman &
Keast, 2013; Running et al., 2015).

![Graph showing detection, recognition and terminal thresholds of tastants.]

**Figure 1.2 – Relationship between chemical concentration, detection threshold and recognition threshold.** The left-hand side represents chemical concentration from 0 M 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.
1.6 Physiological Responses to Oral Fatty Acid Exposure

In humans, a 2.8-fold increase in plasma TG concentrations was recorded in response to oral fat loads (Mattes, 2001a; 2001b). These effects are not observed with sensory-matched fat mimetics, textural cues or smell, supporting the view that fatty acid activates putative taste receptors that generate an immediate signal which is transmitted to other parts of the periphery, preparing the body for fat digestion and absorption. Additional investigations have also reported fat-specific cephalic phase responses following oral stimulation with dietary fat that include increases in lipase secretion (Wøjdemann et al., 1997); transient stimulation of GIT hormones, including cholecystokinin, pancreatic polypeptide and peptide YY (Robertson et al., 2001; Wisén et al., 1992); variations in postprandial glucose and insulin (Robertson et al., 2001; Chavez-Jauregui et al., 2010); as well as increases in satiety (Little & Feinle-Bisset, 2011; Smeets & Westerterp-Plantenga, 2006).

1.7 Relevance of Fat Taste to Development of Obesity

In rodents, differences in fat taste sensitivity appear to influence fat preference, consumption and predisposition to obesity, hinting at a novel role of the taste system in the control of both food intake and weight regulation (Takeda et al., 2001a; 2001b; Verhagen et al., 2003). It has been established that different rodent strains are selectively more or less sensitive to fatty acids and that differences in fat taste are inherently linked to dietary intake and preference. For example, when wild-type mice were compared to FFAR1 and FFAR4 knock-out mice, the knock-out mice showed an attenuated preference for linoleic acid (C18:2) and C18:1, suggesting that FFAR1 and FFAR4 play a role in the perception of fatty acids in mice (Cartoni et al., 2010).
Furthermore, when FFAR4 knock-out mice were fed an *ad libitum* 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). Moreover, an *ad libitum* high-fat diet reduced expression of CD36 in obese rats which may be associated with fat taste adaptation and also indicates a role in regulation of energy intake (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). The above studies suggest a link between oral sensitivity to fatty acids and development of obesity, with animals less sensitive to fatty acids unable to adequately regulate intake and overconsume energy.

A feature of the taste system is the large individual differences in sensitivity to compounds (Keast et al., 2004). Differential dietary practices amongst obese and lean individuals, especially with regard to fat consumption and preference, are also well established, for example obese individuals have shown a higher preference for high-fat foods and greater concentration of fat within specific food matrices when compared to lean individuals (Hooper et al., 2015; Rissanen et al., 2002). However, obesity is multifaceted and complex, and with large variation amongst individuals, proposed contributing factors to obesity may not apply universally to all individuals. Variations in the taste system along with dietary intake and behaviours have been the focus of recent research studies.

The relationship between fat taste sensitivity, dietary fat intake and body mass index (BMI) has been investigated extensively in recent years (Asano et al., 2016; Costanzo et al., 2017; Keast et al., 2014; Kindleysides et al., 2017; Martínez-Ruiz et al., 2014; Stewart & Keast, 2012; Stewart et al., 2010; 2011a; Tucker et al., 2014). In general, it was found that those who were more sensitive to C18:1 had lower energy intakes and consumed less total dietary fat. Some studies also found those who were more
sensitive to C18:1 were also better at detecting the fat content of food (Heinze et al., 2017; Stewart & Keast, 2012; Stewart et al., 2010; 2011a). Another study 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 et al., 2001a). Conversely, hypersensitive individuals reported behaviours including trimming the fat off meat and avoiding saturated fats (Stewart et al., 2011a). Additionally, various human studies have reported that participants who were classified as hypersensitive to fatty acids also had lower BMI than hyposensitive individuals (Asano et al., 2016; Kindleysides et al., 2017; Stewart et al., 2010; 2011a; Martínez-Ruiz et al., 2014; Tucker et al., 2014); however, other studies failed to find such associations (Costanzo et al., 2017; Stewart & Keast, 2012; Running et al., 2013). A recent meta-analysis found no association between fat taste sensitivity and BMI, although the author could not conclude on these findings as it was only based on seven cross-sectional studies (Tucker et al., 2017). It has also been reported that fatty acid sensitivity can be modulated by dietary fat, with a high-fat diet causing attenuation of fat taste thresholds (FTT), while a low-fat diet results in increased FTT (Costanzo et al., 2018; Newman et al., 2016; Stewart & Keast, 2012). Changes in the preference of high-fat foods have been observed when restricting fat intake over 12- to 24-weeks, which led to a decrease in the pleasantness, taste and preference of high-fat foods. This suggests that the experience of fats in foods can be modulated by the diet (Mattes, 1993). However, more recent studies have not been able to replicate changes to preference for high-fat foods after habitual low-fat or high-fat dietary intake (Costanzo et al., 2018; Newman et al., 2016; Stewart & Keast, 2012).
Chapter One: Literature Review

The association between fat taste and obesity is probably a result of a coordinated alimentary canal response to dietary fat (Mattes, 2005; Stewart et al., 2011b). Indeed, a link between oral fatty acid chemoreception and GIT responses to fatty acid has been established with obese individuals having impaired responses to fatty acid in the oral cavity and the GIT (Brennan et al., 2011; Pepino et al., 2012; Samra, 2010; Stewart & Keast, 2012; Stewart et al., 2011b) compared to healthy-weight subjects. The presence of fats in the small intestine in healthy, normal-weight subjects generates potent satiety signals (Stewart et al., 2011b). Gastric emptying is slowed, gut hormones CCK and PYY are released, and ghrelin is inhibited, altogether causing acute suppression of self-reported prospective consumption (Blundell & Macdiarmid, 1997; Feinle et al., 2003). These physiological satiety mechanisms may be impaired in the obese with subjects voluntarily consuming twice as much energy from fat products as non-obese (Blundell et al., 1993; Stewart et al., 2011b). An *ad libitum* breakfast buffet study illustrated the link between fatty acid sensitivity, fat consumption and satiety (Keast et al., 2014). Overall, the high-protein breakfast was the most satiating meal in the study. However, when the population was stratified according to fat taste sensitivity, those who were hyposensitive to C18:1 found the high-fat breakfast the least satiating meal, while those who were classified as hypersensitive to C18:1 found the high-fat breakfast the most satiating meal. This result was specific for the high-fat meal; this was not observed following a high-carbohydrate, high-protein or balanced meal (Keast et al., 2014).
1.8 The Role of Genes on Fat Taste Sensitivity

There is mounting evidence that polymorphisms in the CD36 gene are associated with oral fat perception and fat preference in human subjects. The A allele of single-nucleotide polymorphism (SNP) rs1761667 for CD36 has been associated with reduced fat taste sensitivity, increased creaminess perception, and liking of added fats in foods compared to the G allele in multiple populations (Keller et al., 2012; Melis et al., 2015; Mrizak et al., 2015; Pepino et al., 2012; Sayed et al., 2015). Also, the T allele of SNP rs1527483 for CD36 has been associated with increased perception of fat content compared to the C allele in African-American and Malaysian populations (Keller et al., 2012; Ong et al., 2017), but not in a Caucasian population (Melis et al., 2015). A further nine SNPs for CD36 have been associated with obesity, type 2 diabetes and reduced fat oxidation rate, although these studies did not assess fat taste sensitivity (Liu et al., 2016).

While no study to date has assessed the association between SNPs of the FFAR4 gene and fat taste sensitivity, the G allele of SNP rs116454156 for FFAR4 has been associated with increased obesity and reduced LCFA signal transduction compared to the A allele in a European population (Ichimura et al., 2012). Further, FFAR4 haplotypes T-T-T and T-C-C have been associated with increased BMI in Japanese individuals that have a high dietary fat intake (Waguri et al., 2013). There is potential that these SNPs and haplotypes could be related to impaired fat taste sensitivity, and therefore reduced satiety signalling after oral fat exposure.

There is little information on the heritability of fat taste sensitivity. A familial pedigree analysis determined the variability of C18:2 supra-threshold to be 19% heritable (Garneau et al., 2017). However, as mentioned earlier, supra-threshold is not the most
appropriate indicator of dietary intake as food rarely contains FFA in the supra-threshold range (Che Man et al., 1999; Koriyama et al., 2002), and it is likely that sensory systems other than taste are involved at that level. For reference, a twin study reported the heritability of the variability for detection thresholds to be 22% for salty taste and 53% for sour taste (Wise et al., 2007), providing potential that variation in detection threshold for other tastes may also be, at least in part, heritable.

1.9 Summary

Differences in fat taste sensitivity appear to predict certain dietary behaviours, i.e. decreased sensitivity is associated with an increased consumption of fat, and this has been reported in both animal and human studies. Moreover, sensitivity to fat taste can be modulated by the diet, i.e. consumption of a high-fat diet appears to maximise the body’s capacity for fat intake, suggesting that such changes may accompany or encourage excess fat intake and obesity. These data propose a direct role of the taste system in the consumption of high-fat foods, which may be linked to increased risk of obesity given that differences in BMI have also been linked to oral fatty acid sensitivity. The mechanism allowing for increased consumption of fat is proposed to be via reduced satiety or fullness signals in fat taste impaired individuals, as associations in both taste and digestive responses to fat have been reported.
1.10 Thesis aim, objectives, and hypotheses

1.10.1 Overall Aim and Objectives

The aims of this thesis was to assess whether individual variations in fat taste sensitivity may influence anthropometry and dietary intake (both habitual and acute intake). Further, it aimed to investigate the factors that influence fat taste sensitivity, specifically the role that environment and genetics have on modulating sensitivity; and explore the physiological functions and roles of the fat taste system. More precisely, across the four studies, the objectives were to:

- Assess the associations between FTT, anthropometric measurements, fat intake, TG perception, and liking of fatty foods.
- Assess the effect of low-fat or high-fat dietary intake on FTT, TG perception and liking of fatty foods whilst participants maintain stable weight over a period of 8 weeks.
- Assess the effect of low-fat or high-fat dietary intake on fat taste receptor gene expression in fungiform papillae over a period of 8 weeks.
- Assess the heritability of FTT.
- Explore the effect of genetics on diet-mediated change to FTT.
- Investigate the relative regional sensitivities of papillae clusters to fatty acid.

1.10.2 Hypotheses

This thesis will test the following hypotheses:

- Fat taste sensitivity will be associated with anthropometric measurements, fat intake, TG perception, but not fatty food liking.
• Low-fat dietary intake will reduce FTT and increase TG perception. Conversely, high-fat dietary intake will increase FTT and decrease TG perception. Dietary fat intake will not have an effect on fatty food liking.

• Low-fat dietary intake will increase expression of fat taste receptor genes in fungiform papillae, while high-fat dietary intake will decrease expression.

• A proportion of the variation in FTT will be heritable.

• The circumvallate papillae region will be the most sensitive papillae cluster to fatty acid stimulation.

1.11 Thesis Outline

Chapter One contains an up-to-date review of the literature in this area including background information on the sense of taste and its function; mechanisms for fat taste detection; a summary of criteria for fat taste to be considered a primary taste quality; physiological responses to oral dietary fat exposure; the relevance of fat taste to the development of overweight and obesity; and the role of genetics on fat taste sensitivity.

Chapter Two outlines all methodology used in this research, which comprised four sensory evaluation studies. Chapters Three to Six detail the four studies and include introduction, methods, results, discussion and conclusion for each. Study 1 (Chapter Three) investigated the association between fat taste sensitivity, short-term and habitual dietary intake, obesity, TG perception and liking of fatty foods in Australian adults. Study 2 (Chapter Four) is a co-twin randomised controlled trial (RCT) assessing the effect of dietary fat intake and genetics on fat taste sensitivity. Study 3 (Chapter Five) is a subsample of the co-twin RCT presented in Study 2, assessing the effect of dietary fat intake on fungiform fat taste receptor gene expression, as well
as exploring the association between gene expression and fat taste function. **Study 4** (Chapter Six) investigated the papillae clusters of the tongue that are most susceptible to fatty acid stimulation. Finally, **Chapter Seven** summarises the findings of the four studies conducted, their limitations, and future directions for research.
Chapter Two: Materials, Methodology, and Measurements

Components of this chapter have been published in Nutrients, 2017, 9:781, as ‘Fat Taste Sensitivity Is Associated with Short-Term and Habitual Fat Intake’ and The American Journal of Clinical Nutrition, 2018, in press, as ‘Effect of dietary fat intake and genetics on fat taste sensitivity: a co-twin randomised controlled trial’.

2.1 Introduction

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

2.2 Participants

2.2.1 Recruitment and Demographics

All participants for Study 1, Study 2 and Study 3 were screened prior to study enrolment for eligibility (Appendix A). Participants were not eligible if they: were younger than 18 years or older than 69 years; were pregnant or lactating; were taking any prescription medication that may interfere with their ability to taste; had a history of food allergies that may interfere with these studies; or had special dietary requirements or conditions that may interfere with these studies. Twins Research Australia (TRA) invited via mailout (Appendix B) 1881 twin pairs (3762 individuals)
Chapter Two: Materials, Methodology and Measurements

from the Melbourne metropolitan area, who had not participated in a TRA study in the last 18 months. Reminders were sent to 3430 individuals who did not respond within 3 months via a combination of mailouts, phone calls and emails. A total of 92 participants gave written informed consent and participated in Study 1 and Study 2, and of those participants, a further 26 participants gave written informed consent and participated in Study 3.

Participants for Study 4 were screened prior to study enrolment for eligibility. Participants were not eligible if they: were younger than 18 years or older than 50 years; were underweight (<18.5kg/m²), overweight or obese (>25kg/m²); were pregnant or lactating; or had a history of food allergies that may interfere with these studies. Participants were recruited by flyers (Appendix C) around Deakin University, Burwood, VIC. A total of 20 participants gave written informed consent and participated in Study 4.

2.2.2 Reimbursement

Reimbursement was provided in the form of Coles Myer Group gift cards. A $30 reimbursement was provided for Study 1; a $70 reimbursement was provided for Study 2; a $200 reimbursement was provided for Study 3; and a $60 reimbursement was provided for Study 4.

2.3 Ethics

All studies were conducted according to the guidelines laid down by the Declaration of Helsinki. The Deakin University Human Research Ethics Committee approved all the procedures involving human participants prior to study commencement (Study 1
Chapter Two: Materials, Methodology and Measurements

and Study 2 ID: 2013-110; Study 3 ID: 2013-163; Study 4 ID: 2014-122). Study 2 and Study 3 were registered at www.anzctr.org.au as a clinical trial (ID: ACTRN12613000466741). Written informed consent was obtained from all participants prior to participation and participants were free to withdraw from all studies at any stage throughout the course of the study.

2.4 Sensory Testing

All sensory tasks were conducted in temperature and sound controlled partitioned sensory booths at the Centre for Advanced Sensory Science. Hedonic and intensity ratings were collected using computer software Compusense Cloud as part of the Compusense Academic Consortium (Compusense Inc., Ontario, Canada). Participants were asked to refrain from eating, drinking (except water), chewing gum or brushing for at least one hour prior to testing.

2.4.1 Fat Taste Threshold

Detection threshold to C18:1 (FTT) was measured using established methods (Haryono et al., 2014). Food grade C18:1 (Sigma Aldrich, MO, USA) was stored under nitrogen gas below 4°C. C18:1 was added at varying concentrations (0.02, 0.06, 1.00, 1.40, 2.00, 2.80, 3.80, 5.00, 6.40, 8.00, 9.80, 12.00, and 20.00mM) to long-life fat-free milk (Devondale, VIC, Australia). All preparations were mixed with 5% (w/v) gum arabic (pre-hydrated FT Powder, TIC Gums, NSW, Australia) and 5% (v/v) liquid paraffin (Faulding Remedies, QLD, Australia) to produce perceptually identical textural attributes including viscosity and lubricity between C18:1 and control samples. To prevent oxidation of C18:1, all samples were mixed with 0.01% (w/v)
ethylenediaminetetraacetic acid (Merck, Darmstadt, Germany). Samples were homogenised for 30s/100mL at 12,000rpm (Silverston L4RT homogeniser, MA, USA), prepared no more than 2h prior to testing, and served at room temperature. Control samples were prepared in the same way, but without added C18:1. Participants were asked to rinse their mouths with water before beginning the task and between sample sets. Participants wore nose clips and all tests were conducted under red light.

FTT was determined using ascending series 3-alternate forced choice (3-AFC) methodology (Haryono et al., 2014), which is a test to select a sample among a set of three that differs in a known attribute. In order to familiarise participants with the taste attribute of C18:1 and to reduce sensory fatigue, participants were initially provided with warm-up sets prior to the 3-AFC test. A warm-up set contained a C18:1 sample (initially 3.8mM) and a control sample. If participants were unable to perceive a difference between the control and C18:1 sample during the first warm-up set, then they were provided with a new warm-up set at 8mM.

The 3-AFC test began with the highest C18:1 concentration sample set that could not be differentiated to the control during warm-up (i.e. the 3-AFC test began with 0.02mM if participant was able to differentiate the 3.8mM sample from the control sample). Participants were provided with multiple sample sets each containing three randomly-ordered samples per set, two controls and one containing C18:1. Participants were asked to taste each sample in the set and identify the sample that matched the taste quality from the warm-up sets. Correct identification of the C18:1 sample resulted in the participants repeating the same sample set. Incorrect identification of the C18:1 sample resulted in new sample set with a higher concentration of C18:1. This continues in an ascending order from the initial concentration to the highest concentration (20mM). End-point was defined as the
concentration of C18:1 correctly identified in three consecutive sample sets of the same concentration, in line with commonly established sensory testing procedures (Haryono et al., 2014) (Appendix D). The 3-AFC test was performed in duplicate with participants eating a low-fat plain water cracker (Manassen Foods, NSW, Australia) between tests to reduce sensory fatigue. FTT was defined as the mean of the two endpoints, as two measures on the same day has demonstrated test-retest reliability (Newman & Keast, 2013). If participants were unable to correctly identify the C18:1 sample at the highest concentration for at least one of their trials, then they were given a detection threshold of >20mM. Of note, due to the range of concentrations of fatty acid tested, the outcome FTT is an interval censored variable; i.e., a threshold of 20mM indicates that the participant’s actual threshold is anywhere between 12 and 20mM. FTT was transformed to an ordinal variable, FTT rank, ranging from 0 to 12, with higher ranks implying lower sensitivity (Table 2.1) (Costanzo et al., 2017).

<table>
<thead>
<tr>
<th>Fat Taste Threshold (mM)</th>
<th>Fat Taste Rank</th>
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<tbody>
<tr>
<td>0.02 – 0.99</td>
<td>0</td>
</tr>
<tr>
<td>1.00 – 1.39</td>
<td>1</td>
</tr>
<tr>
<td>1.40 – 1.99</td>
<td>2</td>
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<tr>
<td>2.00 – 2.79</td>
<td>3</td>
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<tr>
<td>2.80 – 3.79</td>
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<td>3.80 – 4.99</td>
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<td>9.80 – 11.99</td>
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<td>12.00 – 19.99</td>
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<td>20.00</td>
<td>11</td>
</tr>
<tr>
<td>&gt;20.00</td>
<td>12</td>
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</tbody>
</table>

Table 2.1 – Fat taste rank and the corresponding fat taste threshold concentration range.
2.4.2 Triglyceride Ranking Task

This task was designed to evaluate ability to discriminate different levels of fat content between food samples (Haryono et al., 2014). Four food samples were made using low-fat custard (Parmalat, QLD, Australia) and varying amounts of canola oil (Woolworths, NSW, Australia) (0%, 2%, 6% and 10% oil \( w/w \)). All samples were stirred vigorously. Custard samples were presented in a randomised order, participants were asked to taste and rank them according to their fat content (Appendix E). The custard samples were served at room temperature and prepared no more than 2h prior to testing. To prevent visual cues, the test was conducted under red light. TG ranking score was calculated using the following formula:

\[
(-2 \times c1) - (1 \times c2) + (1 \times c3) + (2 \times c4)
\]

, where \( c1 \) to \( c4 \) were the concentrations of the samples assigned by the participant as lowest to highest fat content (Bolhuis et al., 2015; De Graaf & Zandstra, 1999). The values of the concentrations were -2 for 0%, -1 for 2%, 1 for 6% and 2 for 10%. Accurately ranking samples in ascending or descending order are both considered to be correct; therefore negative scores were converted to positive values. Final TG ranking score ranged from 0 to 10, with 10 being able to correctly rank the samples based on fat content and 0 being unable.

2.4.3 Hedonic Ratings of Regular-Fat and Reduced-Fat Foods

Liking of food based on fat content was measured by rating seven high-fat (HF) foods and seven reduced-fat (LF) counterparts. Participant liking was measured by rating “liking” on a hedonic labelled magnitude scale (LMS) with anchors −100 (extremely dislike), 0 (neither like nor dislike) and 100 (extremely like) (Appendix F). Subjects
were trained in the use of the hedonic LMS by rating non-taste sensations on a validated liking questionnaire (Duffy et al., 2009) (Appendix G) and were instructed that the ends of the scale were intended for hedonic experiences beyond the context of food. Foods were presented under red light to reduce visual differences between samples. Savoury biscuits (Arnott’s, NSW, Australia), peanut butter (Mondelez, VIC, Australia), hummus (Black Swan, VIC, Australia), salad dressing (Goodman Fielder, NSW, Australia), processed cheese (Mondelez, VIC, Australia), cream cheese (Mondelez, VIC, Australia), and chocolate mousse (Fonterra, VIC, Australia) were tested (Appendix H). Foods were always presented in the order listed above to simulate normal eating behaviour. LF and HF counterparts for each food were presented at the same time, side-by-side, in a randomised order (left or right side).

HF and LF liking scores were calculated as the mean of the seven HF foods and seven LF foods liking ratings, respectively. The differences between the HF and LF scores (HF-LF liking score) was also calculated to control for individual preferences for each food item.

### 2.4.4 Intensity Ratings for Five Prototypical Tastants

Participants rated the intensities of sweet, salty, sour, bitter, and umami solutions at concentrations prepared based on Webb et al. (2015). Concentrations were prepared at supra-threshold concentrations (weak, moderate, and strong) (Appendix I), made using sucrose (Woolworths, NSW, Australia), sodium chloride (Woolworths, NSW, Australia), citric acid (Ward McKenzie, VIC, Australia), caffeine (Sigma-Aldrich, MO, USA) and monosodium glutamate (Ajinomoto Cooperation, Tokyo, Japan), respectively. To prevent confounding non-taste sensory inputs, participants wore nose
clips and the test was conducted under red light. Participants tasted each sample and rated its intensity on an LMS with anchors 0 (no taste) to 100 (strongest imaginable taste) (Appendix J). Subjects were trained in the use of the LMS following published standard procedures (Webb et al., 2015) that involved culturally appropriate remembered or imagined sensations, such as the coolness of an ice-cold beverage, or the sweetness of fairy floss (known as candy floss in the UK, or cotton candy in the USA). All solutions were prepared within five days of testing, stored at 4 °C, and served at room temperature. Participants were presented all three concentrations (weak, moderate and strong) of one taste at a time in random order. The sequence of the tastants was also randomised.

2.4.5 Fatty Acid Exposure to Taste Papillae Regions of the Tongue

A papillae stimulus test was conducted to identify which regions of the tongue are most susceptible to stimulation by C18:1 using a cotton bud. Two stimuli were included in the papillae stimulus test: C18:1 and paraffin oil. Paraffin oil was used as a control due to its absence of FFA, while mimicking the textural properties of C18:1. Participants were asked to protrude their tongue as a researcher administered stimuli on various locations of the tongue using a cotton bud. A stimulus of pure C18:1 or paraffin oil was applied to each papillae region of their tongue (fungiform, foliate and circumvallate; Figure 1), one at a time. Stimulus duration was 2s. After each stimulus application, participants were asked whether they detected any taste sensation (Appendix K). A tongue depressor was used by the researcher to aid in applying stimuli to the circumvallate region of the tongue to prevent a gag-reflex. Stimuli were applied to each side of the tongue (left and right) separately. Applications of the stimuli (stimulus type, papillae region and tongue side) were conducted in a randomized order.
Participants rinsed their mouth with water between each stimulation. A plain, low-fat water cracker was provided halfway through testing (after 6 applications) and participants were given a 2 minute break to prevent sensory fatigue.

2.4.2 Fatty Acid Detection Threshold – Fungiform Papillae

FTT of the fungiform papillae locus (FTT-F) was measured using a solid vehicle to validate the results found in the papillae stimulus test. Only the fungiform locus was measured in this test as foliate and circumvallate testing would cause discomfort in participants. Thresholds were measured using a triangle test with filter paper strips as a vehicle. Food grade C18:1 was added at varying volumes (0.25 to 50µL) to the tasting end of the filter paper strips. Paraffin oil was also added to the tasting end of the filter paper strips so that the total amount of substrate equalled 50µL (e.g., the lowest concentration strip contained 0.25µL C18:1 and 49.75µL paraffin oil). Control strips contained 50µL paraffin oil.

Participants placed the tasting end of the filter paper strips on the tip of the tongue to taste the sample. A diagram was provided to participants to aid in placement of the strip (Appendix L). Samples were presented to the participants and completed in duplicate in the same manner as in the FTT method described in Chapter 2.4.1. The mean of the two final C18:1 volumes was recorded as FTT-F.

2.5 Fat Taste Receptor Gene Expression in Fungiform Papillae

Fungiform papillae biopsy was conducted without anaesthetic by a registered doctor following the procedure described previously (Archer et al., 2016; Spielman et al.,
For each participant, up to 8 fungiform papillae were collected and pooled as an individual sample. Samples were sent to CSIRO for analysis of fat taste receptor gene expression, conducted by researchers independent to this PhD. The expression of fat taste receptor genes including $CD36$, $FFAR2$, $FFAR4$, $GPR84$ and $KCNA2$ were analysed with real time reverse transcription polymerase chain reaction (RT-PCR). Ribonucleic acid (RNA) was extracted from the pooled fungiform papillae samples using TRIZol reagent (Life Technologies, CA, USA) following the manufacture’s protocol. The purified RNA pallet was dissolved in 20μl RNase-free H2O, treated with RNase-free DNase set (Qiagen, VIC, Australia) and quantified with NanoDrop ND-1000 spectrophotometer. The RNA integrity was measured with Bioanalyser 2100 (Agilent Technologies, CA, USA). For the RT-PCR, 1μg of total RNA was used to synthesise complimentary DNA (cDNA) using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, CA, USA). Each cDNA sample was diluted 1:5 at first. Standards were then prepared with a serial dilution 1:5 from the top standard (an aliquot of the all the 1:5 dilution cDNA samples). The expression of the interested genes was analysed with the Taqman gene expression assays (Life Technologies, CA, USA) (Table 2.2). For each gene analysis, a negative control of the sample that had not been reversely transcribed and a positive control from RNA isolated from whole blood were included. Housekeeping genes $GAPDH$ and $RPLP0$ were included for normalising the transcript numbers. Whole blood sample was selected as the positive tissue as all genes analysed were found to be expressed in human whole blood according to the gene expression omnibus profiles of National Centre for Biotechnology Information.
Chapter Two: Materials, Methodology and Measurements

<table>
<thead>
<tr>
<th>Gene</th>
<th>Assay ID</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD36</td>
<td>Hs01567185_m1</td>
<td>Probe spans exons</td>
</tr>
<tr>
<td>FFAR4</td>
<td>Hs00699184_m1</td>
<td>Probe spans exons</td>
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<tr>
<td>FFAR2</td>
<td>Hs00271142_s1</td>
<td>Probes are within single exon</td>
</tr>
<tr>
<td>GPR84</td>
<td>Hs01874713_s1</td>
<td>Probes are within single exon</td>
</tr>
<tr>
<td>KCNA2</td>
<td>Hs04187587_g1</td>
<td>Probe spans exons</td>
</tr>
<tr>
<td>RPLP0</td>
<td>Hs99999902_m1</td>
<td>Probe spans exons</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Hs02758991_m1</td>
<td>Probe spans exons</td>
</tr>
</tbody>
</table>

Table 2.2 – Taqman gene expression assays used for real time RT-PCR analysis.

2.6 Anthropometry

Body weight was measured after removal of shoes, heavy clothing, and any items in their pockets using electronic scales (OHAUS NV4101, Parsippany, NJ, USA). Height was measured using a free-standing stadiometer (SECA, Hamburg, Germany). BMI was calculated as weight (kg)/height (m)^2. Hip and natural waist (midway between the lowest rib and the iliac crest) circumferences were measured according to World Health Organisation guidelines (Gibson, 2005).

2.7 Dietary Intervention

A low-fat diet was defined as <20% of energy from fats and a high-fat diet was defined as >35% of energy from fats. These values were chosen based on previous intervention studies (Newman et al., 2016; Stewart & Keast, 2012). Participants on the high-fat diet were encouraged to choose foods higher in monounsaturated and polyunsaturated fats rather than saturated fats in order to maintain a healthy diet. A diet booklet for each diet was created with the aid of an accredited practising dietitian, which described the parameters of each diet (Appendices N & O); a list of foods which should and should
not be eaten; and some example recipes that adhere to the diet. Participants were given the high-fat or low-fat booklet, their assigned diet was explained and they were taught how to interpret a nutrition information panel in order to identify which foods were acceptable for their diet.

They were requested to start the assigned diet the day after baseline measurement. As foods were not provided in this study, food choice was up to the participants. To maximise adherence to the diets, participants were contacted via phone fortnightly and questioned on their dietary habits. If the researcher felt that participants were not following the diet adequately, they were provided with suggestions and encouragement to aid in diet adherence. Participants were also asked a series of questions to ensure that they did not experience any negative effects from the diet. These questions included “Do you feel like you have less energy since starting the diet?”, “Do you feel like your weight has changed significantly since starting the diet?”, and “Is the diet affecting your day-to-day activities?” If the researcher felt that participants were suffering from major negative effects due to the diet (e.g. severe nausea, inability to work), they would be asked to stop the diet and were dropped out of the trial.

Completed diet diaries, as described below in Chapter 2.6.2, were inspected at 4 weeks and 8 weeks, and reviewed for adherence to the assigned diet.

Participants were encouraged to maintain their baseline weight throughout the study. A target of less than 2kg change in body weight over the trial was set. Participants were asked to stop eating once they were satiated to prevent overconsumption. Weight maintenance guides for each diet (Appendices P & Q), including tips and suggested recipes (e.g., low-fat guide contained low-fat, high-energy recipes to prevent weight
loss), was provided to participants at the start of the trial to help maintain weight. If weight changed more than 1kg from the between baseline and week 4, subjects were given further advice on how to maintain their weight for the latter half of the diet.

2.8 Dietary Assessment

2.8.1 24 Hour Food Recall
A single three-pass 24-hour dietary recall (DoHA, 2010) was collected by a trained nutritionist to assess short-term dietary intake (Appendix R). The food recall was analysed for energy intake (kJ), total consumption (g) of protein, fat (total fat, saturated fat, monounsaturated fat and polyunsaturated fat), carbohydrate and alcohol, and percentage of energy from protein, fat, carbohydrate and alcohol using computer software FoodWorks (version 8, Xyris, QLD, Australia).

Food recalls were assessed for underreporting using Goldberg cut-off values (Goldberg et al., 1991). If the ratio of a participant’s reported energy intake to basal metabolic rate (based on age, sex and weight) was lower than 0.9, then EI was not considered to be plausible and the participant was considered to be a low energy reporter.

2.8.2 24 Hour Food Record
Six 24-hour diet records were completed throughout the dietary intervention – three between baseline and week 4, and three between week 4 and week 8; as three 24-hour diet records are optimal for estimating energy and macronutrient intake (Ma et al., 2009). Diet was recorded for two weekdays and one weekend, chosen by the
participant (Appendix R). Participants were asked to avoid filling out diet records on a non-standard day (for example, if they attended a wedding reception). They were taught to quantify foods in standard serving sizes (cups, teaspoons, tablespoons, etc.) using a food model booklet, and asked to weigh their food and drinks wherever possible. Details such as brand, cooking method, and foods additives (e.g. sugar added to coffee) were included in the diet records. Food records were analysed for energy intake (kJ), total consumption (g) of protein, fat (total fat, saturated fat, monounsaturated fat and polyunsaturated fat), carbohydrate and alcohol, and percentage of energy from protein, fat, carbohydrate and alcohol using computer software FoodWorks.

2.8.3 Food Frequency Questionnaire

A Food Frequency Questionnaire (FFQ) adapted from the 1995 Australian National Nutrition Survey FFQ (McLennan & Podger, 1999) was used to assess habitual pattern of food intake (Appendix S). Participants were required to indicate on average, how many times in the previous month they consumed 96 different food or beverage items. In total, nine 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; and fruits. The frequency they could be consumed ranged from ‘never or less than once a month’ to ‘six or more times per day’. Each frequency category was converted into a daily equivalent value for occasions of consumption: 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 (Newman, 2013).
For analysis, food items were categorised into food groups based on the classification system used in the 2011-2013 Australian Health Survey (ABS, 2013), which used a hierarchical numeric system to classify foods. In this system, individual food and beverage items are assigned an eight-digit food ID where two- and three-digit food groups describe major and sub-major food groups, respectively. All food items from the FFQ were categorised based on two-digit food codes, except for dairy foods which were categorised based on three-digit food codes to differentiate high-fat and low-fat sources of dairy.

The following major food groups were combined into a ‘meat & meat alternatives’ category to reduce multiple comparisons, as they all contain significant amounts of dietary protein: ‘meat, poultry and game products and dishes’, ‘fish and seafood products and dishes’, ‘egg products and dishes’, ‘seed and nut products and dishes’ and ‘dairy and meat substitutes’. Dairy products were split into two categories: low-fat dairy and high-fat dairy based on their ‘sub-major food groups’. Any foods flagged as ‘discretionary’ by the Australian Health Survey – Discretionary Food List were categorised into a ‘discretionary foods’ category and not included in any other food group (ABS, 2013). The final categories analysed included meat & meat alternatives, fruits, vegetables, high-fat dairy, low-fat dairy, grains & cereals, discretionary foods, and alcoholic beverages. The full categorisation of each food item is listed in Appendix T.

2.9 Statistical Analyses
The statistical approach used in each study is described in detail in the corresponding chapter. In all studies, numerical variables are reported as mean and standard deviation
(SD), and categorical data as \( n \) and percent (%). Null hypotheses were rejected at \( P < 0.05 \). Model assumptions were checked on the residuals of the model. If model assumptions were broken, non-parametric models were used where appropriate. In Study 1, Study 2 and Study 3, the statistical approach for the analyses always accounted for the fact that data were collected in twins. Linear mixed models were conducted including twin pair as a random effect to account for the clustering of co-twins. Additionally, in Study 2 and Study 3, the repeated measured structure of the data was considered through modelling the covariance matrix.
Chapter Three: Study 1 – Fat Taste Sensitivity Is Associated with Short-Term and Habitual Fat Intake

This study has been published in Nutrients, 2017, 9:781 as ‘Fat Taste Sensitivity Is Associated with Short-Term and Habitual Fat Intake’.

3.1 Introduction

Dietary fat is an energy-dense macronutrient that contributes to approximately 31–32% of energy intake in Australian adult diets (ABS, 2015). Passive overconsumption of dietary fats is common due to their palatability, which is a major contributor to the development of overweight and obesity. Oral perception of fatty acids has recently been recognised as a primary taste, also known as fat taste, which may have a regulatory role in the consumption of dietary fat in humans (Keast & Costanzo, 2015; Mattes, 2011; Running et al., 2015). For fat taste to be initiated, fatty acid must activate fat taste receptors located on taste cells (Liu et al., 2016). FFA occur in small amounts in fatty foods (Koriyama et al., 2002), and human lingual lipases may also increase fatty acid exposure in the oral cavity by hydrolysing TG (Pepino et al., 2012). In large amounts, fatty acids elicit a rancid taste to prevent the consumption of spoiled lipids in foods. It should be noted that fat taste differs from TG perception, which imparts odour and textural dimensions presumably independent of the fat taste dimension.
Cross-sectional studies have shown that fat taste sensitivity is associated with the consumption of fatty foods, such that those with a lower sensitivity tend to consume larger amounts of dietary fat. Furthermore, there is some evidence that those with a lower sensitivity to fat taste are more likely to have a higher BMI (Asano et al., 2016; Martínez-Ruiz et al., 2014; Stewart et al., 2010; Stewart et al., 2011a; Stewart et al., 2011b). However, the link between fat taste and BMI is contentious, as many studies have been unable to find an association (Bolhuis et al., 2015; Running et al., 2013; Stewart & Keast, 2012; Tucker et al., 2015). A recent meta-analysis of seven cross-sectional studies found that sensitivity to fat taste does not contribute to or result from obesity (Tucker et al., 2017).

FTT is the current standard for measuring sensitivity to fat taste, in that sensitivity decreases as FTT increases (Heinze et al., 2015). FTT is defined as the lowest amount of fatty acid necessary to produce a detectable taste response. There are large variations in FTT among the population (Running et al., 2013; Stewart & Keast, 2012) possibly due to variations in habitual fat intake. Two intervention studies aiming to assess the link between fat intake and FTT both showed that FTT decreased when participants were exposed to a low-fat diet over a four-week (Stewart & Keast; 2012) and six-week (Newman et al., 2016) period. In addition, FTT increased in healthy weight participants exposed to a high-fat diet over a four-week period (Stewart & Keast; 2012), but there was no increase in FTT in overweight or obese individuals, presumably because their fat taste sensitivity was already impaired. However it remains uncertain whether the change in thresholds are specific to total fat intake or related to weight fluctuations as participants in both studies did not maintain their baseline weight, losing weight on the low-fat diet and gaining weight on the high-fat diet (Newman et al., 2016; Stewart & Keast; 2012).
The ability to discriminate foods based on perception of TG also has health implications as it can allow individuals to make food choices which are lower in dietary fats. TG perception varies widely amongst the population (Liang et al., 2012; Stewart et al., 2011a) and limited evidence suggests an association between fat taste sensitivity and TG perception (Stewart & Keast, 2012; Stewart et al., 2010; 2011a;). Since fat taste is only the perception of fatty acid in the oral cavity, this association is likely to be unrelated to TG itself, but may instead be due to naturally occurring FFA in fatty foods (Koriyama et al., 2002) or hydrolysis of TG into fatty acid by lingual lipases (Pepino et al., 2012). Similarly, obese individuals (Pepino & Mennela, 2014) or individuals with a larger waist circumference (Liang et al., 2012) have been found to have impaired perception of TG compared to healthy weight individuals.

Liking fatty foods is also a promoter of dietary fat consumption (Martínez-Ruiz et al., 2014), although the current evidence for the relationship between fat perception and health outcomes is weak (Cox et al., 2016). There may be a relationship between liking of fatty foods and fat taste sensitivity, with sensitive individuals preferring low-fat foods compared to less sensitive individuals (Bolhuis et al., 2015; Martínez-Ruiz et al., 2014). This is likely due to the unpleasant rancid taste of FFA found in some high-fat foods (Koriyama et al., 2002).

### 3.2 Aims and Hypotheses

#### 3.2.1 Aims

The aims of this study were to assess the associations between FTT, anthropometric measurements, fat intake, and liking of fatty foods in a sample of Australian adults.
3.2.2 Hypotheses

- FTT will be positively associated with fat intake.
- FTT will not be associated with anthropometric measures or liking of fatty foods.

3.3 Participants, Materials, and Methods

3.3.1 Participants

Sixty-six twin pairs (132 individuals) were screened by TRA, as described in Chapter 2.2.1, to participate in an RCT assessing the impact of alterations in dietary fat intake and heritability on fat taste function (Study 2). Monozygotic (MZ) and dizygotic (DZ) pairs were included in this study. Participants were eligible if 18 to 69 years old, did not have dairy allergies or intolerances or illnesses preventing them from eating foods included in the study, and they were neither pregnant nor lactating. Forty-six twin pairs (34 female pairs, 11 male pairs, and one gender discordant pair; 92 individuals) were eligible for the RCT and completed baseline measurements.

The study was conducted in accordance with the Declaration of Helsinki, the protocol was approved by the Deakin University Human Research Ethics Committee (2013-110), and written informed consent was obtained by all participants prior to participation.

3.3.2 Study Outline

Each participants attended a 2h laboratory session at the Centre for Advanced Sensory Science at Deakin University, Burwood, VIC. Tests were conducted in a temperature and sound controlled partitioned sensory booths using computer software Compusense Cloud as part of the Compusense Academic Consortium. A 15 minute break was
provided in the middle of each session to prevent fatigue. Participants were asked to avoid eating or drinking anything but water and to avoid brushing their teeth or using mouthwash up to an hour prior to their tasting session. Tasting sessions measured for: FTT; TG ranking score; liking ratings for high-fat and reduced-fat foods; and intensity ratings to five prototypical tastants (Haryono et al., 2014). Anthropometric measurements were at the beginning of the taste session. A 24-hour food recall was collected during the session by a nutritionist. Approximately one week prior to the tasting session, participants were asked to complete an FFQ on their eating habits, which was completed at home.

3.3.3 Fat Taste Threshold

FTT was measured using established methods (Haryono et al., 2014), as described in Chapter 2.4.1. An ascending series 3-AFC methodology was used, where participants were provided with multiple sample sets each containing three randomly-ordered samples of fat-free milk per set, two controls and one containing C18:1 at varying concentrations (0.02, 0.06, 1.00, 1.40, 2.00, 2.80, 3.80, 5.00, 6.40, 8.00, 9.80, 12.00, and 20.00mM). Participants were asked to taste each sample in the set and identify the sample that matched the taste quality from the warm-up sets, described further in Chapter 2.4.1. Correct identification of the C18:1 sample resulted in the participants repeating the same sample set. Incorrect identification of the C18:1 sample resulted in new sample set with a higher concentration of C18:1. FTT was defined as the concentration of C18:1 correctly identified in three consecutive sample sets of the same concentration. FTT was transformed to an ordinal variable — FTT rank — ranging from 0 to 12, with higher ranks implying lower sensitivity to fat taste (Table 2.1).
3.3.4 Triglyceride Ranking Task

The TG ranking task evaluated the ability to discriminate foods based on fat content, as described in Chapter 2.4.2. Canola oil was added to low-fat custard up to 2%, 6% and 10% \((w/w)\) oil in custard. A custard sample remained oil free (0%). All samples were stirred vigorously. All four custard samples were presented to participants in a randomised order, and participants were asked to taste each sample and rank them according to their fat content.

3.3.5 24 Hour Food Recall

A 24-hour food recall was used to assess short-term dietary intake, as described in Chapter 2.7.1. Food recalls were analysed for energy intake (MJ), total consumption (g) of protein, fats (total fat, saturated fat, monounsaturated fat, and polyunsaturated fat), carbohydrates, and alcohol, and percentage of energy derived from protein, fats, and carbohydrates using computer software FoodWorks.

3.3.6 Hedonic Ratings of Regular-Fat and Reduced-Fat Foods

Liking of foods based on fat content was measured rating seven HF foods and seven LF counterparts, as described in Chapter 2.4.3. Liking was measured by rating “liking” on a hedonic LMS with from −100 to 100. HF and LF liking scores were calculated as the mean of the seven HF foods and seven LF foods liking ratings, respectively. The differences between the HF and LF scores (HF-LF liking score) was also calculated to control for individual preferences for each food item.
3.3.7 Intensity Ratings for Five Prototypical Tastants

Participants rated the intensities of sweet, salty, sour, bitter, and umami solutions at concentrations prepared based on Webb et al. (2015), as described in Chapter 2.4.4. Concentrations were prepared at supra-threshold concentrations (weak, moderate, and strong). Participants tasted each sample and rated the intensities on a LMS from 0 to 100.

3.3.8 Anthropometry

Body weight, height, BMI, waist circumference and hip circumference were measured as described in Chapter 2.6.

3.3.9 Food Frequency Questionnaire

A FFQ adapted from the 1995 Australian National Nutrition Survey FFQ (McLennan & Podger, 1999) was used to assess habitual pattern of food intake, as described in Chapter 2.7.3. Participants were required to indicate on average, how many times in the previous month they consumed 96 different food or beverage items. Food items were categorised into food groups based on the classification system used in the 2011–2013 Australian Health Survey (ABS, 2013). The final categories analysed included meat & meat alternatives, fruits, vegetables, high-fat dairy, low-fat dairy, grains & cereals, discretionary foods, and alcoholic beverages.

3.3.10 Statistical Analysis

Statistical analyses were conducted using computer software SAS (v9.3, SAS Institute, NC, USA). Null hypotheses were rejected at $P < 0.05$. Descriptive statistics
Chapter Three: Study 1 – Fat Taste Sensitivity Is Associated with Short-Term and Habitual Fat Intake

are reported as mean and SD, and categorical data presented as \( n \) and %. Estimated coefficients obtained under linear mixed-effects models are reported along with 95% confidence intervals. Estimated coefficients obtained under linear mixed-effects models (\( \hat{\beta} \)) are reported along with 95% confidence intervals. \( \hat{\beta} \) indicates the change in FTT rank per unit of change in the independent variable after controlling for other variables in the model.

The association between FTT rank and other variables was assessed using linear mixed-effects models including the variable of interest and age as fixed effects, and the twin pair as a random effect to account for the clustering induced by the twin. We included age as a covariate to account for any effect of age on taste. When considering associations between FTT rank and nutrient intakes, we report the estimates from the model described above (unadjusted) and from a model with energy adjusted FTT rank to control for differences in energy intake (Goldberg et al., 1991). We also report associations between FTT rank and nutrient energy as a percentage of total energy intake. FFQ food group analyses were also conducted with and without adjusting for energy intake. The associations between FTT rank and nutrient intakes, as above, were also compared between underweight/healthy weight individuals and overweight/obese individuals using the same linear mixed model including BMI status as a fixed effect.

3.4 Results

The main outcome variable, FTT rank, was extremely skewed in males, with 10 of 23 men classified in the two more extreme categories, while FTT rank in females had a uniform distribution (Figure 3.1). This skewed distribution in males breaks
Chapter Three: Study 1 – Fat Taste Sensitivity Is Associated with Short-Term and Habitual Fat Intake

assumptions of the linear mixed model. We have therefore reported female and male results separately; the male results presented for descriptive purposes only.

Figure 3.1 – Distribution of FTT rank for male and female participants in Study 1.

Characteristics of the participants are detailed in Table 3.1. For FTT, all inter-individual 3-AFC test end-points were within three concentration steps apart for the first two measurements. Therefore, no individuals in this study needed to complete a third 3-AFC test.
Chapter Three: Study 1 – Fat Taste Sensitivity Is Associated with Short-Term and Habitual Fat Intake

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Males (n = 23)</th>
<th>Females (n = 69)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46.4 (16.7)</td>
<td>41.3 (15.6)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175.7 (7.8)</td>
<td>163.1 (7.8)</td>
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<tr>
<td>Weight (kg)</td>
<td>85.2 (15.7)</td>
<td>70.1 (16.7)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.7 (5.0)</td>
<td>26.3 (5.7)</td>
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<td>Waist Circumference (cm)</td>
<td>94.5 (14.1)</td>
<td>82.2 (15.3)</td>
</tr>
<tr>
<td>Waist-Hip Ratio</td>
<td>0.92 (0.08)</td>
<td>0.80 (0.07)</td>
</tr>
<tr>
<td>Fat Taste Rank</td>
<td>8.5 (3.5)</td>
<td>6.3 (3.4)</td>
</tr>
<tr>
<td>Weight Status</td>
<td>n (%)</td>
<td></td>
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<td>Underweight</td>
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<tr>
<td>Healthy Weight</td>
<td>7 (30%)</td>
<td>33 (48%)</td>
</tr>
<tr>
<td>Overweight</td>
<td>11 (48%)</td>
<td>13 (19%)</td>
</tr>
<tr>
<td>Obese</td>
<td>5 (29%)</td>
<td>20 (29%)</td>
</tr>
</tbody>
</table>

Table 3.1 – Characteristics of participants in Study 1. Underweight BMI ≤ 18.5kg/m²; healthy weight BMI = 18.5 to 24.9kg/m²; overweight BMI = 25 to 29.9kg/m²; obese BMI ≥ 30kg/m².

3.4.1 24 Hour Food Recall

Sixteen females were classified as low energy reporters according to Goldberg cut-off values (Goldberg et al., 1991). No males were classified as low energy reporters. The following analyses in females were conducted with and without low energy reporters. Estimated coefficients and conclusions were similar regardless of low energy reporter exclusion. Therefore, we report the analyses using the entire sample. Energy and nutrient intakes of the participants who were assessed in this study are detailed in Table 3.2.

In females, there was no significant association between FTT rank and energy intake (MJ) (Table 3.3). After adjusting for energy intake, there was a significant positive association between FTT rank and percent (%) energy from fat (P = 0.044), and a significant negative association between % energy from carbohydrate (P = 0.004).
Chapter Three: Study 1 – Fat Taste Sensitivity Is Associated with Short-Term and Habitual Fat Intake

This indicates, for example, a 10% increase in energy from total fat would be associated with a 1.10 unit change in FTT rank. In addition, there were positive associations that approached significance between FTT rank, % energy from monounsaturated fat ($P = 0.067$) and % energy from saturated fat ($P = 0.100$). In males, there was a significant association between FTT rank and energy intake (MJ) ($P = 0.017$), total fat intake (g) ($P = 0.036$) and saturated fat intake (g) ($P = 0.015$) (Table 3.3). After adjusting for energy intake, there was only a significant positive association between FTT rank and percent (%) energy from saturated fat.

When assessing participants split by BMI status group (underweight/healthy weight versus overweight/obese), there was no significant effect of BMI status on the associations between FTT rank and any of the macronutrient intakes in either gender.

### 3.4.2 Food Frequency Questionnaire

The FFQ assessed self-reported habitual food consumption of specific food items. In females, after adjusting for energy intake, there were significant positive associations between FTT rank and daily consumption of meat & meat alternatives ($P = 0.009$), HF dairy ($P = 0.043$) and grain & cereal ($P = 0.007$) (Table 3.4). In males, there was a significant positive association between FTT rank and daily consumption of alcoholic beverages ($P = 0.041$), but not after adjusting for energy intake.
Chapter Three: Study 1 – Fat Taste Sensitivity Is Associated with Short-Term and Habitual Fat Intake

Females ($n = 69$) | Males ($n = 23$)
---|---
**Nutrient Intake (g)** | **% Energy from Nutrients** | **Nutrient Intake (g)** | **% Energy from Nutrients**
Energy (MJ) | 7.8 (2.8) | - | 11.8 (4.9) | -
Total Fat | 73.5 (33.4) | 34.2 (7.5) | 113.8 (72.5) | 33.9 (8.8)
Sat. Fat | 28.1 (13.8) | 13.0 (3.6) | 47.9 (34.2) | 14.2 (4.4)
Mono. Fat | 28.2 (13.8) | 13.1 (3.6) | 41.1 (26.1) | 12.2 (3.9)
Poly. Fat | 11.0 (7.9) | 5.2 (2.6) | 15.7 (11.3) | 4.7 (2.4)
Protein | 90.7 (36.9) | 20.2 (5.3) | 125.7 (48.6) | 18.7 (5.6)
CHO | 189.4 (76.5) | 40.3 (9.1) | 280.9 (130.6) | 38.2 (11.6)
Alcohol | 4.7 (13.9) | 1.7 (5.2) | 15.2 (31.0) | 4.9 (11.3)

Table 3.2 – Energy and nutrient intakes from the 24-hour food recall. Data presented as mean (SD). Sat. Fat, saturated fat; Mono. Fat, monounsaturated fat; Poly. Fat, polyunsaturated fat; CHO, carbohydrate.
## Chapter Three: Study 1 – Fat Taste Sensitivity Is Associated with Short-Term and Habitual Fat Intake

### Nutrient Intake

<table>
<thead>
<tr>
<th>Nutrient Intake: Adjusted for Energy</th>
<th>% Energy from Nutrients</th>
<th>Nutrient Intake: Adjusted for Energy</th>
<th>% Energy from Nutrients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Females (n = 69)</strong></td>
<td></td>
<td><strong>Males (n = 23)</strong></td>
<td></td>
</tr>
<tr>
<td>Energy (MJ)</td>
<td>0.1 (-0.2, 0.4)</td>
<td>-</td>
<td>0.3 (0.1, 0.5)*</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>0.02 (-0.01, 0.04)</td>
<td>0.02 (-0.01, 0.04)</td>
<td>0.02 (0.00, 0.03)*</td>
</tr>
<tr>
<td>Sat. Fat (g)</td>
<td>0.05 (-0.02, 0.11)</td>
<td>0.05 (-0.01, 0.12)</td>
<td>0.20 (-0.04, 0.45)</td>
</tr>
<tr>
<td>Mono. Fat (g)</td>
<td>0.04 (-0.02, 0.10)</td>
<td>0.04 (-0.01, 0.10)</td>
<td>0.22 (-0.02, 0.45)</td>
</tr>
<tr>
<td>Poly. Fat (g)</td>
<td>-0.01 (-0.13, 0.12)</td>
<td>-0.01 (-0.13, 0.12)</td>
<td>0.06 (-0.42, 0.55)</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>0.01 (-0.01, 0.03)</td>
<td>0.01 (-0.01, 0.03)</td>
<td>0.08 (-0.07, 0.23)</td>
</tr>
<tr>
<td>CHO (g)</td>
<td>0.00 (-0.01, 0.01)</td>
<td>0.00 (-0.01, 0.00)</td>
<td>-0.11 (-0.19, -0.04)**</td>
</tr>
<tr>
<td>Alcohol (g)</td>
<td>0.02 (-0.02, 0.06)</td>
<td>0.02 (-0.02, 0.06)</td>
<td>0.08 (-0.03, 0.18)</td>
</tr>
</tbody>
</table>

**Table 3.3 – Associations between fat taste rank, energy, and macronutrient intakes from the 24-hour food recall.** Data presented as $\hat{\beta}$ (95% CI). $\hat{\beta}$, estimated coefficient obtained under a linear mixed model including twin pair as a random effect; regression analysis was adjusted for age; Sat. Fat, saturated fat; Mono. Fat, monounsaturated fat; Poly. Fat, polyunsaturated fat; CHO, carbohydrate; * $P$-value < 0.05; ** $P$-value < 0.01.
Table 3.4 – Associations between fat taste rank and frequency of food group consumption as reported by the Food Frequency Questionnaire. Data presented as $\hat{\beta}$ (95% CI). $\hat{\beta}$, estimated coefficient obtained under a linear mixed model including twin pair as a random effect; regression analysis was adjusted for age; HF, high-fat; LF, low-fat; * P-value < 0.05; ** P-value < 0.01.
3.4.3 Hedonic Ratings of Regular-Fat and Reduced-Fat Foods

Liking scores of the seven LF and HF foods (savoury biscuits, peanut butter, hummus, salad dressing, processed cheese, cream cheese, and chocolate mousse) were rated from -100 to 100. LF and HF liking scores were calculated as the mean of the seven LF and HF food liking ratings, respectively. In females, the mean LF liking score was 13.6 (11.3), HF liking score was 20.6 (11.6) and the difference (HF-LF) liking score was 7.0 (7.6). In males, the mean LF liking score was 9.6 (11.5), HF liking score was 16.3 (12.6) and HF-LF liking score was 6.7 (7.7). There were no significant associations between FTT rank and LF liking score ($\beta = 0.03$ [95% CI: -0.04, 0.09]), HF liking score ($\beta = 0.00$ [-0.06, 0.05]) or HF-LF liking score ($\beta = -0.01$ [-0.02, 0.01]) in females, or between FTT rank and LF liking score ($\beta = -0.12$ [-0.27, 0.03]) in males. However, in males, there were significant negative associations between FTT rank and HF liking score ($\beta = -0.17$ [-0.26, -0.08], $P < 0.001$), and HF-LF liking score ($\beta = -0.03$ [-0.05, -0.01], $P = 0.001$).

3.4.4 Triglyceride Ranking Task

The possible scores for the fat ranking task ranged from 0–10, with 10 being able to fully discriminate the fat content between the samples from lowest to highest. The mean score for the fat ranking task was 6.1 (3.8) in females and 5.1 (3.4) in males. No association was observed between fat ranking task score and FTT rank in females ($\beta = -0.05$ [-0.28, 0.18]) or males ($\beta = -0.14$ [-0.65, 0.38]).
Chapter Three: Study 1 – Fat Taste Sensitivity Is Associated with Short-Term and Habitual Fat Intake

3.4.5 Intensity Ratings for Five Prototypical Tastants

No significant associations were observed between FTT rank and sensitivity to any of the five basic tastes in females (sweet: $\hat{\beta} = 0.18 [-2.22, 2.97]$; salty: $\hat{\beta} = 0.14 [-2.68, 2.97]$; sour: $\hat{\beta} = -1.45 [-4.70, 1.81]$; bitter: $\hat{\beta} = -2.20 [-5.05, 0.66]$; umami: $\hat{\beta} = -0.51 [-3.14, 2.12]$). In males, there were significant positive associations between FTT rank and sensitivity to sweet ($\hat{\beta} = 3.83 [1.23, 6.42], P = 0.004$), sour ($\hat{\beta} = 4.02 [0.58, 7.47], P = 0.022$) and umami ($\hat{\beta} = 3.36 [0.46, 6.25], P = 0.023$), but not salty ($\hat{\beta} = 1.77 [-3.03, 6.57]$) and bitter ($\hat{\beta} = 1.00 [-2.64, 4.65]$).

3.4.5 Anthropometry

There were no associations between FTT rank and anthropometric measurements in females (BMI: $\hat{\beta} = 0.08 [-0.17, 0.33]$; waist circumference: $\hat{\beta} = 0.04 [-0.03, 0.11]$; waist-hip ratio: $\hat{\beta} = 0.49 [-12.67, 13.65]$) or males (BMI: $\hat{\beta} = 0.07 [-0.35, 0.48]$; waist circumference: $\hat{\beta} = 0.02 [-0.11, 0.15]$; waist-hip ratio: $\hat{\beta} = 0.14 [-19.55, 19.83]$).

3.5 Discussion

The current study assessed the associations between FTT rank, anthropometric measurements, fat intake, and liking of fatty foods in healthy Australian adults. However, the distribution of FTT rank in males was heavily skewed to the right, which is likely due to the small number of males in the study. Linear mixed models assume normal distribution of a sample, therefore we could not make conclusions based on the male results. While males, on average, consume more dietary fat than females (ABS, 2015), the underlying mechanisms that control fat taste sensitivity are not likely
Chapter Three: Study 1 – Fat Taste Sensitivity Is Associated with Short-Term and Habitual Fat Intake

to be influenced by gender. Therefore, this discussion will focus on the results from the female data.

There were no associations between FTT rank, anthropometric measurements and liking of fatty foods. There were positive associations between FTT rank and fat intake, only when expressed as % energy from fat, and a negative association between FTT rank and % energy from carbohydrate. Habitual consumption of meat & meat-alternatives, high-fat dairy, and grain & cereal were positively associated with FTT rank. There was no association between FTT rank and total dietary fat intake (g), with or without controlling for energy. This indicates that fat taste sensitivity is associated with the proportion of fat consumed relative to total energy intake rather than the total amount of fat consumed. It should be noted that the nutrient intakes in this sample was reflective of Australian diets, as energy from macronutrients were similar to what was found in the Australian Health Survey 2011–2012 (ABS, 2015).

The literature surrounding the relationship between fat taste sensitivity and adiposity is mixed. Many studies have reported a negative association between fat taste sensitivity and BMI (Asano et al., 2016; Martínez-Ruiz et al., 2014; Stewart et al., 2010; 2011a; 2011b) while others reported no association (Bolhuis et al., 2015; Running et al., 2013; Stewart & Keast, 2012; Tucker et al., 2015). Methodologies used in these studies are similar, and reasons for the differing results are not clear. A recent meta-analysis of seven cross-sectional studies clearly demonstrated that fat taste sensitivity was not associated with BMI (Tucker et al., 2017). The results from the current study match the meta-analysis, in that BMI was not associated with fat taste sensitivity. Similarly, we found no association between fat taste sensitivity and waist circumference or waist-hip ratio.

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Despite impaired fat taste sensitivity having no association with BMI, it may still have implications for negative health outcomes. The current Australian and international dietary recommendations are to reduce saturated fat intake and increase polyunsaturated fat intake (NHMRC, 2006; WHO, 2007). In the current study, associations between FTT rank, % energy of saturated, and % energy of monounsaturated fat were in the expected direction (positive), although they did not reach statistical significance. Tucker et al. (2014) previously reported a correlation between saturated fat and fat taste sensitivity, although not for monounsaturated fat. The associations between FTT rank and polyunsaturated fat, after adjusting for both intake and percentage of energy, were negligible. If impaired fat taste sensitivity does contribute to increased saturated fat intake, then understanding the factors that influence fat taste sensitivity is important.

The food groups associated with fat taste sensitivity were also assessed in this study. Frequency of consumption of foods per day from high-fat dairy, grain & cereal, and meat & meat alternative food groups, was associated with higher FTT rank. Increasing the consumption of high-fat dairy, grain & cereal, or meat & meat alternative foods in one occasion per day is associated with increases of 1.1, 0.8, and 0.7 units in FTT rank, respectively. The large increase in FTT rank associated with consumption of high-fat dairy matches the macronutrient intake data from this study, as high-fat dairy is a major source of saturated and monounsaturated fat. However, the association with meat & meat alternatives is harder to understand, as there was no association between FTT rank and protein intake. It is likely that the association between FTT rank and meat & meat alternatives is due to fat in meat products. While the Australian Guide to Healthy Eating (NHMRC, 2006) recommends consumption of lean sources of protein, the FFQ used was not able to differentiate whether meat items was lean or not.
example, “Mixed dishes with beef, veal—e.g., casserole, stir-fry”). Therefore, we could not split meat & meat alternatives into high-fat and low-fat categories as we did with dairy foods. However, previous research has shown that regularly trimming fat off meat prior to consumption is positively associated with fat taste sensitivity (Stewart et al., 2011a). The association between FTT rank and grain & cereal consumption is also difficult to understand because there was a negative association between FTT rank and carbohydrate intake. In an effort to understand this, we assessed the different food items that fell into this category to see if any of the items were responsible for this association. Of the 9 food items, only “white bread, toast or rolls” had a significant association with FTT rank ($\beta = 1.623 \ [0.243, 3.003] \ P < 0.05$). We assume that this association may be due to the addition of butter and other fatty spreads that are commonly consumed with white bread, and not due to the bread itself. This is supported by Stewart et al. (2011a), as it was found that consumption of high fat spreads was negatively associated with fat taste sensitivity. When “white bread, toast, or rolls” is omitted from the grain & cereal category, it is no longer significantly associated with FTT rank.

In the current study, it was found that liking fatty foods is not associated with fat taste sensitivity. This has been demonstrated in previous studies (Stewart & Keast, 2012; Newman et al., 2016), although there is some evidence that less sensitive individuals have a higher preference for fatty foods (Asano et al., 2016; Bolhuis et al., 2015). This is interesting, as it suggests that hedonics are not drivers for fat intake with regard to fat taste sensitivity. Similarly, fat perception had no association with fat taste sensitivity. It is likely that the main driver of fat intake in less sensitive individuals is a reduced satiety response to fatty foods, both from impaired taste signalling and reduced GIT response (Stewart et al., 2011b).
There are limitations that should be acknowledged for this study. As this is a secondary analysis of baseline data of the main trial, sample size was calculated for the trial, and therefore the study is underpowered to detect small associations between BMI and FTT rank. However, our sample size is comparable to other similar studies that assessed the association between fat taste sensitivity and BMI (Asano et al., 2016; Bolhuis et al., 2015; Stewart et al., 2010, 2011a; Tucker et al., 2014). Macronutrient intake assessment was based on one 24-hour recall prior to the day of testing. A 24-hour recall is subject to memory and participant biases, and it only provides an estimate of one day’s intake. Lastly, the FFQ was not sufficiently detailed to enable the discrimination of lean or fatty types of meat.

### 3.6 Conclusion

Evidence from literature surrounding fat taste sensitivity and adiposity, food consumption, hedonics, and TG perception is mixed. The current study is supportive of findings from recent studies regarding fat taste, in that there is no association between fat taste sensitivity and BMI, hedonics, and TG perception, although short-term and habitual fat intake is associated with higher fat taste sensitivity, particularly saturated and monounsaturated fat.
Chapter Four: Study 2 – Effect of Dietary Fat Intake and Genetics on Fat Taste Sensitivity: a Co-Twin Randomised Controlled Trial

This study has been accepted for publication in The American Journal of Clinical Nutrition, 2018, as ‘Effect of dietary fat intake and genetics on fat taste sensitivity: a co-twin randomised controlled trial’.

4.1 Introduction

Individuals with impaired oral fatty acid sensitivity (fat taste) may be more likely to consume greater amounts of fatty foods, mainly from foods high in saturated and monounsaturated fat (Costanzo et al., 2017; Keast & Costanzo, 2015; Mattes, 2011; Running et al., 2015). Impaired sensitivity to fat taste is paralleled by impaired satiety responses to fatty acids in the GIT, which could lead to increased consumption of dietary fat (Bolhuis et al., 2015; Keast et al., 2014; Stewart et al., 2011b). In addition, impaired sensitivity to fat taste has been shown to be associated with increased liking, preference and choice for fatty foods (Bolhuis et al., 2015; Martínez-Ruiz et al., 2014). Habitual fat intake is responsible for variation in fat taste, with increasing dietary fat causing increased FTT. While cross-sectional studies have shown positive associations between fat intake and FTT (Costanzo et al., 2017; Stewart et al., 2010; 2011b; Tucker et al., 2015), intervention studies have demonstrated that modifications to long-term dietary fat intake mediates change in FTT. Two dietary fat cross-over
intervention studies of 4-week (Stewart & Keast, 2012) and 6-week (Newman et al., 2016) duration, reported decreased FTT after low-fat dietary intake (Newman et al., 2016; Stewart & Keast, 2012) and increased FTT after high-fat dietary intake albeit only in lean individuals (Stewart & Keast, 2012). However, in both studies, participants on the low-fat diet lost significant weight over the intervention period (Newman et al., 2016; Stewart & Keast, 2012), and participants on the high-fat diet gained significant weight (Stewart & Keast, 2012). Authors could not rule out the possibility that at least part of the reported effect was due to weight differences, especially considering that in one of the studies the effect was only evident in lean individuals. However, body weight does not seem to be associated with FTT, as a recent meta-analysis of seven cross-sectional studies found no relationship between fat taste sensitivity and obesity (Tucker et al., 2017).

Two variants of the $CD36$ gene have been associated with fat taste sensitivity. The A allele of single-nucleotide polymorphism (SNP) $rs1761667$ for $CD36$ was associated with reduced fat taste sensitivity and increased creaminess perception and liking of added fats in foods compared to the G allele in multiple populations (Keller et al., 2012; Melis et al., 2015; Mrizak et al., 2015; Pepino et al., 2012). Also, the T allele of SNP $rs1527483$ for $CD36$ was associated with increased perception of fat content compared to the C allele in an African-American population (Keller et al., 2012), but not in a Caucasian population (Melis et al., 2015). However, it is not known whether SNPs of these genes regulate changes in fat taste sensitivity after dietary intervention. Genetic components of a phenotype can be assessed by comparing monozygotic (MZ) and dizygotic (DZ) twin pairs under the assumption that MZ pairs share 100% of their genes and DZ pairs share, on average, 50% of their genes (Neale & Maes, 2004).
4.2 Aims & Hypotheses

4.2.1 Aims

The aim of this study was to assess the effect of low-fat (<20% energy from fat) or high-fat (>35% energy from fat) dietary intake on FTT whilst recommending participants maintain stable weight over 8 weeks. This study also aims to assess heritability of FTT at baseline, and to explore the effect of genetics on diet-mediated FTT changes by assessing whether zygosity modifies the diet effect. Secondary aims were to assess the effect of low-fat or high-fat dietary intake on TG perception, liking of fatty foods and intensity ratings to five prototypical tastants. A co-twin design was chosen as it controls for age, and genetic and common environmental factors shared by co-twins in each experimental group.

4.2.2 Hypotheses

- Low-fat dietary intake will reduce FTT and increase TG perception, and high-fat dietary intake will increased FTT and decrease TG perception.
- A proportion of the variation in FTT will be heritable, and genetics will have an effect on diet-mediated change to FTT.
- Dietary fat intake will not have an effect on fatty food liking or sensitivity to the prototypical tastants.
4.3 Participants, Materials, and Methods

4.3.1 Participants

Twins were eligible to participate in the study if they were aged between 18-69 years, were able to attend three laboratory sessions in Burwood, VIC, and were willing to alter their diet for a period of 8 weeks. Both MZ and DZ twin pairs were invited to participate. Subjects were excluded from recruitment if they had any dairy allergies and intolerances, illnesses preventing them from eating foods included in the study, or if they were pregnant or lactating. Due to the nature of the twin study design, if one individual from a twin pair was excluded or withdrew from the study, their co-twin was also excluded.

Sixty-six twin pairs (132 individuals) were screened by TRA to participate in this study, as described in Chapter 2.2.1. Forty-six twin pairs (92 individuals) aged between 18-68 years were recruited into the study, and co-twins from each pair were randomised into either a low-fat or high-fat diet. Prior to recruitment, a block randomised sequence was generated with blocks of size two. TRA was responsible for recruitment and therefore characteristics of the participants were blinded to the researchers. Participants were allocated to the randomised sequence based on their TRA twin number; therefore, allocation of participants to diet group was concealed. Due to the nature of the intervention, blinding of participants was not feasible. Ethics was approved by the Deakin University Human Research Ethics Committee (2013-110) in accordance with the Declaration of Helsinki, and informed written consent was obtained by all participants prior to participation. This study was registered at www.anzctr.org.au as a clinical trial (ID: ACTRN12613000466741).
4.3.2 Study Outline
Participants attended three 2h laboratory sessions at the Centre for Advanced Sensory Science at Deakin University, Burwood, VIC. Sessions were conducted 4 weeks apart (Figure 4.1). Tests were conducted in a temperature and sound controlled environment with a 15 minute break in the middle of their session to prevent fatigue. Participants were asked to avoid eating or drinking anything but water and to avoid brushing their teeth or using mouthwash up to an hour prior to each tasting session. Tasting sessions measured for detection thresholds to oleic acid; TG ranking score; liking ratings for high-fat and reduced-fat foods; and intensity ratings to five prototypical tastants (Haryono et al., 2014). Liking and intensity ratings were collected using computer software Compusense Cloud as part of the Compusense Academic Consortium. Anthropometric measurements were taken at the beginning of each session. A 24-hour food recall was collected by a nutritionist during the first session. Between tasting sessions, participants recorded three 24-hour diet records (2 weekdays, 1 weekend day) to determine dietary compliance.

4.3.3 Dietary Intervention
The low-fat diet was defined as <20% of energy from fats and the high-fat diet was defined as >35% of energy from fats, as described in Chapter 2.7. Participants were requested to start the assigned diet the day after baseline measurement. As foods were not provided in this study, food choice was up to the participants. To maximise adherence to the diets, participants were contacted via phone fortnightly and questioned on their dietary habits, described in detail in Chapter 2.7. Food records, as described in Chapter 2.8.2, were inspected at the beginning of sessions 2 and 3, and reviewed for adherence to the assigned diet.
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Figure 4.1 – Outline of Study 2 timeline. Food records collected on 2 weekdays and 1 weekend day at any time during weeks 0-4 and week 4-8. 24-hour dietary recall was not repeated at session 2 and 3.
Participants were encouraged to maintain their baseline weight throughout the study. A target of less than 2kg change in body weight over the trial was set. Participants were asked to stop eating once they were satiated to prevent overconsumption. Weight maintenance guides for each diet, including tips and suggested recipes (e.g., low-fat guide contained low-fat, high-energy recipes to prevent weight loss), was provided to participants at the start of the trial to help maintain weight. If weight changed more than 1kg from the between baseline and week 4 (session 2), subjects were given further advice on how to maintain their weight for the latter half of the study.

4.3.4 Dietary Assessment
A single three-pass 24-hour dietary recall of the day prior to session 1 was conducted by a trained nutritionist to assess short-term dietary intake, as described in Chapter 2.8.1. Participants also completed three 24-hour diet records between session 1 & 2; and between session 2 & 3 (Figure 4), as described in Chapter 2.8.2.

4.3.5 Anthropometry
Body weight, height, BMI, waist circumference and hip circumference were measured as described in Chapter 2.6.

4.3.6 Fat Taste Threshold
FTT was measured using established methods (Haryono et al., 2014), as described in Chapter 2.4.1. An ascending series 3-AFC methodology was used, where participants were provided with multiple sample sets each containing three randomly-ordered
samples of fat-free milk per set, two controls and one containing C18:1 at varying concentrations (0.02, 0.06, 1.00, 1.40, 2.00, 2.80, 3.80, 5.00, 6.40, 8.00, 9.80, 12.00, and 20.00mM). Participants were asked to taste each sample in the set and identify the sample that matched the taste quality from the warm-up sets, described further in Chapter 2.4.1. Correct identification of the C18:1 sample resulted in the participants repeating the same sample set. Incorrect identification of the C18:1 sample resulted in a new sample set with a higher concentration of C18:1. FTT was defined as the concentration of C18:1 correctly identified in three consecutive sample sets of the same concentration. FTT was transformed to an ordinal variable — FTT rank — ranging from 0 to 12, with higher ranks implying lower sensitivity to fat taste (Table 2.1).

4.3.7 Triglyceride Ranking Task

The TG ranking task evaluated the ability to discriminate foods based on fat content, as described in Chapter 2.4.2. Canola oil was added to low-fat custard up to 2%, 6% and 10% (w/w) oil in custard. A custard sample remained oil free (0%). All samples were stirred vigorously. All four custard samples were presented to participants in a randomised order, and participants were asked to taste each sample and rank them according to their fat content.

4.3.8 Hedonic Ratings of Regular-Fat and Reduced-Fat Foods

Liking of foods based on fat content was measured rating seven HF foods and seven LF counterparts, as described in Chapter 2.4.3. Liking was measured by rating “liking” on a hedonic LMS with from −100 to 100.
HF and LF liking scores were calculated as the mean of the seven HF foods and seven LF foods liking ratings, respectively. The differences between the HF and LF scores (HF-LF liking score) was also calculated to control for individual preferences for each food item.

### 4.3.9 Intensity Ratings to Five Prototypical Tastants

Participants rated the intensities of sweet, salty, sour, bitter, and umami solutions at concentrations prepared based on Webb et al. (2015), as described in 2.4.4. Concentrations were prepared at supra-threshold concentrations (weak, moderate, and strong). Participants tasted each sample and rated the intensities on a LMS from 0 to 100.

### 4.3.10 Statistical Analysis

Numerical variables are reported as mean and standard deviation (SD), and categorical data as $n$ and percent (%). Null hypotheses were rejected at $P < 0.05$.

The effect of the diet on anthropometry, dietary compliance, FTT rank, TG ranking score, food liking, and intensity ratings to five prototypical tastants was assessed using linear mixed models including diet (low-fat and high-fat), time (baseline, week 4 and week 8) and time-diet interaction as fixed effects; with twin pair as a random effect to account for the correlation between co-twins, and participant as the subject with repeated measures. The same analysis was repeated for FTT rank in the subset of participants whose weight changed less than 2kg. Post-hoc Sidak’s $P$-values confidence intervals are reported.
Further analysis explored the effect of genetics on diet-mediated changes in FTT rank, the only variable that showed significant diet effect using the linear mixed model described above, further including zygosity (MZ and DZ) and all double and triple interactions. The effect of the diet on FTT rank for MZ and DZ pairs is reported at each time. A greater diet effect in DZ pairs compared to MZ pairs would suggest some degree of genetic effect that regulates changes to FTT.

To explore the strength of the association ($\beta$) between change in FTT rank and overall change in fat intake (g) and energy from fat (%) over the 8 week period, a linear mixed model was used including $\Delta$ FTT rank ($\Delta$, week 8 – baseline) as the outcome and $\Delta$ fat intake or $\Delta$ energy from fat as a fixed effect; with twin pair as a random effect. The Pearson’s correlation ($r$) between $\Delta$ FTT rank and $\Delta$ fat intake is also reported for descriptive purposes.

Twin concordance for anthropometric measures and FTT rank was estimated through the intraclass correlation coefficient (ICC) separately for MZ and DZ twin pairs. ICC was additionally estimated controlling for co-twin living status (together or apart). A Wald test was used to compare MZ and DZ pair ICC estimates (Self & Liang, 1987). Correlation between twin pairs is assumed to be due to two factors, an additive genetic effect (A) and a common environmental effect shared by the twin pair (C), with residual variance of an individual attributed to unique environment effects (E) (Neale & Maes, 2004). These effects can be calculated to provide an estimate of heritability, which is the degree of variation of a phenotype that is attributable to additive genetic effects (Neale & Maes, 2004). Heritability ($h^2$) of baseline FTT rank was estimated using a variance components model including zygosity as a fixed effect and twin pair as a random effect. Under the assumption that individual variance can be modelled as
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\[ \sigma^2 = A + C + E, \text{ MZ covariance as } \sigma^2_{\text{MZ}} = A + C, \text{ and DZ covariance as } \sigma^2_{\text{DZ}} = \frac{A}{2} + C \]

since DZ pairs share 50% of genes (Fisher, 1918; Neale & Maes, 2004; Rabe-Hesketh et al., 2008), heritability can be estimated as \( h^2 = \frac{A}{A+C+E} \).

Analyses were conducted using computer software SPSS (v22.0, IBM, NY, USA), except for twin concordance and heritability analyses which were conducted using computer software STATA (v15.0, StataCorp, TX, USA).

4.3.11 Sample Size Calculation

Data from an unpublished non-twin dietary intervention informed the sample size calculations. Computer software PASS (v15.0, NCSS, UT, USA) was used to calculate sample size and power estimates. A sample size of 38 subjects per diet group achieves 82% power to detect a mean difference in FTT of 5.5mM C18:1 between diet groups when the standard deviation of change is 8.5mM C18:1 and the correlation between co-twins is 0.1 (significance level 0.05, two-sided two-sample paired means analysis using simulation). Under the same assumptions, a correlation between co-twins of 0.3 or 0.5 achieves a power of 92% and 97%, respectively. In addition, the target sample size (38 pairs) achieves 85% power for detecting a FTT change of 3mM C18:1 between baseline and week 8 in one of the groups assuming a standard deviation of 6mM C18:1 (significance level 0.05, two-sided one-sample t-test). Forty-six pairs were recruited to allow for 15% attrition.

To assess if 34 MZ and 10 DZ pairs from the final sample had adequate power to estimate heritability for baseline FTT rank, we performed a post-hoc sample size calculation to detect A and C for the equation \( h^2 = \frac{A}{A+C+E} \). The calculations are based
on the proportion of MZ twin pairs in the sample. The ideal proportion of MZ pairs in a sample is 0.61 for detecting A (Visscher, 2004) and 0.06 for detecting C (Visscher et al., 2008), whereas the proportion of MZ pairs in this study was 0.77. To detect the contribution of additive genetic effects (A) to a trait with 80% power required at least 16 MZ pairs and 10 DZ pairs, and to detect the contribution of common environmental effects (C) to a trait with 80% power required at least 10 MZ pairs and 149 DZ pairs. Since this study was only powered to estimate A, we present the heritability estimate for baseline FTT rank but not the full ACE model.

4.4 Results

Sixty-six pairs of twins expressed interest in participating and 46 pairs (70%) were eligible and randomised into the study (Figure 4.2). Two twin pairs dropped out of the study after baseline measurements. One female individual dropped out due to difficulty adhering to the low-fat diet, while another male individual on the high-fat diet did not give a reason for dropping out. Accordingly their co-twins were excluded from the study. The trial was completed by 44 twin pairs (35 MZ, 9 DZ; 33 female pairs, 10 male pairs, 1 gender-discordant pair). There were 34 females (77.3%) in the low-fat diet group and 33 females (75%) in the high-fat diet group. The mean age for both groups was 43.7 (15.5) years.
4.4.1 Anthropometry

There was a significant time-diet interaction for both weight and BMI (Table 4.1). There was a significant decrease in weight and BMI from baseline to week 4 and from baseline to week 8 in the low-fat group. There were no significant changes observed in weight, BMI, waist circumference or waist-hip ratio in the high-fat group over the 8 weeks. At baseline, the high-fat group had a significantly higher waist-hip ratio than the low-fat group. However, this was no longer significantly different between groups after baseline. No other between-group differences were observed over the trial.
### Table 4.1 – Low-fat and high-fat diet within- and between-group mean differences in anthropometrical measurements over the 8-week trial.

Baseline data presented as mean (SD); all differences presented as mean and 95% CI. Between-group differences calculated as high-fat diet – low-fat diet; means, CIs and \( P \)-values estimated under a linear mixed model including diet, time, and time-diet interaction as fixed effects; with twin pair as a random effect to account for the correlation between co-twins, and participant as the subject with repeated measures. Post-hoc Sidak’s test and CIs are reported. WC, waist circumference; WHR, waist-hip ratio; * \( P \)-value < 0.05, *** \( P \)-value < 0.001

<table>
<thead>
<tr>
<th></th>
<th>Low-Fat Diet ( n=44 )</th>
<th>High-Fat Diet ( n=44 )</th>
<th>Between-Group Difference</th>
<th>Time-Diet Interaction ( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Weight (kg)</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>Baseline</td>
<td>72.6 (16.9)</td>
<td>74.7 (18.1)</td>
<td>2.1 (-1.9, 6.1)</td>
<td></td>
</tr>
<tr>
<td>Week 4 vs. Baseline</td>
<td>-0.9 (-1.4, -0.4)***</td>
<td>0.2 (-0.4, 0.7)</td>
<td>3.1 (-1.0, 7.3)</td>
<td></td>
</tr>
<tr>
<td>Week 8 vs. Baseline</td>
<td>-1.3 (-1.9, 0.6)***</td>
<td>0.1 (-0.6, 0.7)</td>
<td>3.4 (-0.8, 7.7)</td>
<td></td>
</tr>
<tr>
<td><strong>BMI (kg/m^2)</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>Baseline</td>
<td>26.3 (5.1)</td>
<td>26.8 (5.8)</td>
<td>0.5 (-0.7, 1.7)</td>
<td></td>
</tr>
<tr>
<td>Week 4 vs. Baseline</td>
<td>-0.3 (-0.5, -0.1)***</td>
<td>0.0 (-0.1, 0.2)</td>
<td>0.9 (-0.4, 2.1)</td>
<td></td>
</tr>
<tr>
<td>Week 8 vs. Baseline</td>
<td>-0.5 (-0.7, -0.2)***</td>
<td>0.0 (-0.2, 0.2)</td>
<td>1.0 (-0.3, 2.3)</td>
<td></td>
</tr>
<tr>
<td><strong>WC (cm)</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.767</td>
</tr>
<tr>
<td>Baseline</td>
<td>83.6 (15.4)</td>
<td>86.7 (16.1)</td>
<td>3.1 (-0.7, 6.9)</td>
<td></td>
</tr>
<tr>
<td>Week 4 vs. Baseline</td>
<td>-0.8 (-2.4, 0.7)</td>
<td>-1.0 (-2.6, 0.5)</td>
<td>2.9 (-1.1, 6.9)</td>
<td></td>
</tr>
<tr>
<td>Week 8 vs. Baseline</td>
<td>-0.7 (-2.3, 0.9)</td>
<td>-0.1 (-1.8, 1.4)</td>
<td>3.6 (-0.2, 7.5)</td>
<td></td>
</tr>
<tr>
<td><strong>WHR</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.529</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.814 (0.09)</td>
<td>0.838 (0.09)</td>
<td>0.025 (0.000, 0.049)*</td>
<td></td>
</tr>
<tr>
<td>Week 4 vs. Baseline</td>
<td>0.003 (-0.013, 0.018)</td>
<td>-0.005 (-0.020, 0.011)</td>
<td>0.017 (-0.007, 0.042)</td>
<td></td>
</tr>
<tr>
<td>Week 8 vs. Baseline</td>
<td>-0.001 (-0.016, 0.013)</td>
<td>-0.011 (-0.026, 0.003)</td>
<td>0.015 (-0.006, 0.035)</td>
<td></td>
</tr>
</tbody>
</table>
Chapter Four: Study 2 – Effect of Dietary Fat Intake and Genetics on Fat Taste Sensitivity: a Co-Twin Randomised Controlled Trial

There were 21 individuals who were not able to maintain baseline body weight (±2.0kg) over the 8-week trial. In the low-fat diet group, 11 individuals lost more than 2kg and 1 gained more than 2kg. In the high-fat diet group, 4 individuals lost more than 2kg and 5 gained more than 2kg.

4.4.2 Fat Taste Threshold

Compared with baseline, in the low-fat group FTT rank significantly decreased from 6.8 to 3.6 at week 4 (-3.2 [-4.3, -2.0], \(P < 0.001\)) and to 2.6 at week 8 (-4.2 [-5.4, -3.0], \(P < 0.001\)) in the low-fat group. In the high-fat group FTT rank significantly increased from 6.9 to 8.3 at week 8 (+1.4 [0.2, 2.6], \(P = 0.017\)), but not at week 4. There was a significant between-group difference at week 4 (4.3 [3.3, 5.4], \(P < 0.001\)) and at week 8 (5.7 [4.6, 6.9], \(P < 0.001\)) (Figure 4.3). There was a significant time-diet interaction (\(P < 0.001\)).

The analysis of FTT rank was also conducted including only participants who maintained body weight (low-fat: \(n = 32\); high-fat: \(n = 35\)) (Figure 4.3). The change in FTT rank in this subgroup was similar to the full analysis.
Figure 4.3 – Means and 95% CI for fat taste rank by diet group over 8-week trial. The analysis on the left includes all participants, while the analysis on the right includes only participants who were able to maintain baseline weight (within ±2.0kg) over the trial. Means, CIs and $P$-values estimated under a linear mixed model including diet, time, and time-diet interaction as fixed effects; with twin pair as a random effect to account for the correlation between co-twins, and participant as the subject with repeated measures. Time-diet interaction for both analyses $P < 0.001$. † indicates within-group difference from baseline; † $P$-value < 0.05, ††† $P$-value < 0.001 * indicates between-group differences; *** $P$-value < 0.001
We explored the association between change in FTT rank and change in total dietary fat intake over the 8 weeks. The estimated association between Δ FTT rank (Δ, week 8 – baseline) and Δ fat intake (g) was $\hat{\beta} = 0.041 \ (0.034, 0.048], P < 0.001, r = 0.475)$ (Figure 4.4) and between Δ FTT rank and Δ energy from fat (%) was $\hat{\beta} = 0.15 \ (0.12, 0.19], P < 0.001)$. This means that for every 10g fat intake or 1% change in energy from fat, FTT rank changed in the same direction by 0.41 or 0.15 respectively.

Figure 4.4 – Δ fat taste rank vs. Δ fat intake at week 8. Slope ($\hat{\beta}$) and $P$-value estimated under a linear mixed model including Δ FTT rank as the outcome and Δ fat intake as a fixed effect; with twin pair as a random effect; $r$ estimated using Pearson’s correlation for descriptive purposes. Circle markers (○) indicate participants in the low-fat group ($n = 44$); square markers (□) indicate participants in the high-fat group ($n = 44$). $\hat{\beta} = 0.041, P < 0.001, r = 0.475$.

4.4.3 Genetic Variation on Fat Taste Threshold

All twins in this study were reared together. At the time of the study, 11 pairs lived at the same address (7 MZ; 4 DZ) and 33 pairs lived apart (27 MZ; 6 DZ). Mean baseline within-pair difference and ICC estimates, a measure of co-twin correlation, of each
zygosity group are detailed in Table 4.2. For the sake of comparison, we include the analysis for anthropometric measures alongside FTT rank. As expected, MZ pairs had a significantly higher ICC than DZ pairs for all anthropometric measurements. However, the ICC estimated for FTT rank was low for both MZ and DZ pairs, with no difference between zygosity. Additionally, when controlling for co-twin living status (together or apart), ICC estimates for FTT rank remained similar (MZ ICC: 0.306; DZ ICC: 0.278). Baseline FTT rank heritability was estimated as 8%.

<table>
<thead>
<tr>
<th></th>
<th>MZ pairs (n = 34)</th>
<th>DZ pairs (n = 10)</th>
<th>Comparison of ICC P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Within-Pair</td>
<td>ICC</td>
<td>Within-Pair</td>
</tr>
<tr>
<td>FTT rank</td>
<td>3.1 (2.8)</td>
<td>0.334</td>
<td>2.8 (2.7)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>1.9 (1.4)</td>
<td>0.972</td>
<td>6.0 (9.2)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>5.7 (6.3)</td>
<td>0.859</td>
<td>17.1 (16.4)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>2.0 (2.2)</td>
<td>0.852</td>
<td>4.7 (4.2)</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>7.0 (6.4)</td>
<td>0.798</td>
<td>14.3 (14.6)</td>
</tr>
<tr>
<td>WHR</td>
<td>0.05 (0.05)</td>
<td>0.679</td>
<td>0.09 (0.09)</td>
</tr>
</tbody>
</table>

Table 4.2 – Mean within-pair differences and intraclass correlations of twin pairs for each zygosity at baseline. Larger ICC in MZ pairs than DZ pairs suggests some genetic contribution to a trait under the assumption that MZ twins share 100% genes and DZ twins share ~50% genes. Absolute within-pair difference values reported as mean (SD). P-values were obtained using a Wald test to compare MZ and DZ ICC. MZ, Monozygotic; DZ, Dizygotic; ICC, Intraclass Correlation Coefficient; WC, Waist Circumference; WHR, Waist-Hip Ratio; FTT rank, Fat Taste Rank

Effect modification by zygosity was explored by comparing the diet effect on FTT rank in MZ pairs and DZ pairs. The greater the difference of the diet effect on FTT rank between DZ pairs and MZ pairs, the greater degree genetic effects have on regulating changes to FTT. The effect of the diet was not significantly different between zygosities at any timepoint (Table 4.3), and there was no evidence of time-
diet-zygosity interaction \((P = 0.892)\), i.e. – the pattern of FTT rank in each diet group was similar for MZ and DZ pairs (Figure 4.5).

<table>
<thead>
<tr>
<th></th>
<th>MZ pairs (n = 34)</th>
<th>DZ pairs (n = 10)</th>
<th>Time-Diet-Zygosity Contrasts</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fat Taste Rank</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.00 (-1.54, 1.4)</td>
<td>0.40 (-2.43, 3.23)</td>
<td>0.40 (-2.84, 3.64)</td>
</tr>
<tr>
<td>Week 4</td>
<td>4.23 (3.05, 5.41)</td>
<td>4.63 (2.37, 6.89)</td>
<td>0.40 (-2.17, 2.96)</td>
</tr>
<tr>
<td>Week 8</td>
<td>5.56 (4.34, 6.78)</td>
<td>6.65 (4.12, 9.18)</td>
<td>1.09 (-1.73, 3.92)</td>
</tr>
</tbody>
</table>

Table 4.3 – Mean diet effect on fat taste rank by zygosity and time. Greater differences within DZ pairs compared to MZ pairs indicate greater genetic contribution to diet-mediated change in fat taste rank. Mean and 95% CI. Estimates and CIs obtained under a linear mixed model including diet, time, zygosity, and all double and triple interactions as fixed effects. MZ, monozygotic; DZ, dizygotic.

Figure 4.5 – Means and 95% CI for fat taste rank by diet group and zygosity. Means, CIs and \(P\)-values estimated under a linear mixed model including diet, time, zygosity, and all double and triple interactions as fixed effects. Time-diet-zygosity interaction \(P = 0.892\). MZLF, low-fat monozygotic; MZHF, high-fat monozygotic; DZLF, low-fat dizygotic; DZHF, high-fat dizygotic.
4.4.4 Dietary Compliance

Compared with baseline, there was a significant reduction in energy (kJ) intake in the low-fat group at week 4 and week 8, and a significant increase in energy intake in the high-fat group at week 8 (Table 4.4). Energy intake was significantly higher in the high-fat group than the low-fat group at week 4 and 8.

Total fat (g), saturated fat (g), monounsaturated fat (g), polyunsaturated fat (g) and protein (g) were significantly different between diet-groups at week 4 and 8 (Table 4.4 & 4.5). The low-fat group participants significantly reduced their intake of total fat, saturated fat, monounsaturated fat and polyunsaturated fat at week 4 and week 8; while the high-fat group significantly increased their intake of total fat and monounsaturated fat at week 4 and week 8, and intake of saturated fat and polyunsaturated fat only at week 4.

As expected, percentage of energy from total fat, saturated fat, monounsaturated fat, polyunsaturated fat, protein and carbohydrate differed significantly between groups at week 4 and week 8 (Table 4.4 & 4.5). The low-fat group significantly decreased energy derived from total fat, saturated fat, monounsaturated fat and polyunsaturated fat, and significantly increased energy derived from protein and carbohydrate at week 4 and week 8. The high-fat group significantly increased energy derived from total fat and monounsaturated fat at week 4 and week 8. Mean percentage of energy from total fat in the low-fat group was 16.7% and 17.2% at week 4 and week 8, respectively. Similarly, mean percentage of energy from total fat in the high-fat group was 39.6% and 38.4% at week 4 and week 8, respectively. Both diet groups complied in average with the required levels of energy from fat, less than 20% and more than 35%.
### Table 4.4 – Low-fat and high-fat diet within- and between-group mean differences in energy, protein, carbohydrate, and alcohol intake in Study 2.

Baseline data presented as mean (SD); all differences presented as mean and 95% CI. Between-group differences calculated as high-fat diet – low-fat diet; Means, CIs and P-values estimated under a linear mixed model including diet, time, and time-diet interaction as fixed effects; with twin pair as a random effect to account for the correlation between co-twins, and participant as the subject with repeated measures. Post-hoc Sidak’s test and CIs are reported. *P*-value <0.05; **P*-value <0.01; ***P*-value <0.001

<table>
<thead>
<tr>
<th></th>
<th>Low-Fat Diet ( (n = 44) )</th>
<th>High-Fat Diet ( (n = 44) )</th>
<th>Between-Group Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy (kJ)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>9076 (4293)</td>
<td>8375 (3224)</td>
<td>-700 (-1907, 506)</td>
</tr>
<tr>
<td>Week 4 vs. Baseline</td>
<td>-2810 (-7079, -1541)***</td>
<td>1276 (-3, 2555)</td>
<td>3385 (2546, 4225)***</td>
</tr>
<tr>
<td>Week 8 vs. Baseline</td>
<td>-2159 (-3335, -983)***</td>
<td>1257 (75, 2440)*</td>
<td>2716 (1682, 3750)***</td>
</tr>
<tr>
<td><strong>Protein</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>100.5 (45.4)</td>
<td>98.3 (39.1)</td>
<td>-2.2 (-17.3, 13.0)</td>
</tr>
<tr>
<td>Week 4 vs. Baseline</td>
<td>-12.7 (-27.5, 2.1)</td>
<td>8.3 (-6.6, 23.3)</td>
<td>18.8 (8.5, 29.2)**</td>
</tr>
<tr>
<td>Week 8 vs. Baseline</td>
<td>-9.5 (-24.9, 5.9)</td>
<td>5.5 (-10.0, 21.0)</td>
<td>12.9 (2.4, 23.4)*</td>
</tr>
<tr>
<td><strong>Carbohydrate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>222.7 (105.9)</td>
<td>193.5 (90.8)</td>
<td>-29.2 (-63.8, 5.4)</td>
</tr>
<tr>
<td>Week 4 vs. Baseline</td>
<td>-25.4 (-57.9, 7.1)***</td>
<td>17.5 (-15.3, 50.3)</td>
<td>13.7 (-8.0, 35.4)</td>
</tr>
<tr>
<td>Week 8 vs. Baseline</td>
<td>8.7 (-25.9, 43.3)</td>
<td>28.9 (-5.7, 63.6)</td>
<td>-9.0 (-53.2, 35.2)</td>
</tr>
<tr>
<td><strong>Alcohol</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>7.2 (16.5)</td>
<td>8.2 (23.0)</td>
<td>1.0 (-7.6, 9.6)</td>
</tr>
<tr>
<td>Week 4 vs. Baseline</td>
<td>-2.9 (-10.6, 4.7)</td>
<td>-3.3 (-11.1, 4.4)</td>
<td>0.6 (-4.1, 5.4)</td>
</tr>
<tr>
<td>Week 8 vs. Baseline</td>
<td>-4.4 (-11.0, 2.1)</td>
<td>0.4 (-6.1, 7.0)</td>
<td>5.9 (-4.3, 16.1)</td>
</tr>
</tbody>
</table>
Table 4.5 – Low-fat and high-fat diet within- and between-group mean differences in dietary fat intake in Study 2. Baseline data presented as mean (SD); all differences presented as mean and 95% CI. Between-group differences calculated as high-fat diet – low-fat diet; Means, CIs and P-values estimated under a linear mixed model including diet, time, and time-diet interaction as fixed effects; with twin pair as a random effect to account for the correlation between co-twins, and participant as the subject with repeated measures. Post-hoc Sidak’s test and CIs are reported. * P-value < 0.05; ** P-value < 0.01; *** P-value < 0.001; Mono, Monounsaturated; Poly, Polyunsaturated.
4.4.5 Triglyceride Ranking Task

There was no significant time-diet interaction for TG ranking score (Table 4.6).

4.4.6 Hedonic Ratings of Regular-Fat and Reduced-Fat Foods

There was no significant time-diet interaction for LF liking score, HF liking score or HF-LF liking score, although we observed significant within-group changes to HF liking score and HF-LF liking score (Table 4.6).

4.4.7 Intensity Ratings to Five Prototypical Tastants

There were no significant time-diet interactions for intensity ratings to any of the prototypical tastants ($P > 0.05$), although we observed significant within-group changes to sweet and bitter intensity ratings (Table 4.7).
### Table 4.6 – Low-fat and high-fat diet within- and between-group mean differences in triglyceride ranking score and food liking scores over the 8-week trial.

Baseline data presented as mean (SD); all differences presented as mean and 95% CI. Between-group differences calculated as high-fat diet – low-fat diet; means, CIs and P-values estimated under a linear mixed model including diet, time, and time-diet interaction as fixed effects; with twin pair as a random effect to account for the correlation between co-twins, and participant as the subject with repeated measures. Post-hoc Sidak’s test and CIs are reported. LF, low fat; HF, high fat; TG, triglyceride; * P-value < 0.05

<table>
<thead>
<tr>
<th></th>
<th>Low-Fat Diet</th>
<th>High-Fat Diet</th>
<th>Between-Group Differences</th>
<th>Time-Diet Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 44)</td>
<td>(n = 44)</td>
<td></td>
<td>(P-value)</td>
</tr>
<tr>
<td><strong>TG Ranking Score</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.849</td>
</tr>
<tr>
<td>Baseline</td>
<td>6.3 (3.5)</td>
<td>5.6 (3.9)</td>
<td>-0.7 (-2.2, 0.8)</td>
<td></td>
</tr>
<tr>
<td>Week 4 vs. Baseline</td>
<td>-0.1 (-1.9, 1.8)</td>
<td>0.1 (-1.7, 2.0)</td>
<td>-0.5 (-1.9, 1.0)</td>
<td></td>
</tr>
<tr>
<td>Week 8 vs. Baseline</td>
<td>-0.5 (-2.3, 1.4)</td>
<td>-0.9 (-2.7, 1.0)</td>
<td>-1.1 (-2.6, 0.4)</td>
<td></td>
</tr>
<tr>
<td><strong>LF Liking Score</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.757</td>
</tr>
<tr>
<td>Baseline</td>
<td>13.8 (10.8)</td>
<td>11.0 (12.1)</td>
<td>-2.9 (-6.3, 0.6)</td>
<td></td>
</tr>
<tr>
<td>Week 4 vs. Baseline</td>
<td>0.0 (-3.0, 3.0)</td>
<td>1.3 (-1.6, 4.3)</td>
<td>-1.6 (-4.7, 1.6)</td>
<td></td>
</tr>
<tr>
<td>Week 8 vs. Baseline</td>
<td>0.4 (-2.5, 3.3)</td>
<td>1.5 (-1.4, 4.4)</td>
<td>-1.7 (-5.2, 1.8)</td>
<td></td>
</tr>
<tr>
<td><strong>HF Liking Score</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.645</td>
</tr>
<tr>
<td>Baseline</td>
<td>19.9 (11.5)</td>
<td>18.9 (12.4)</td>
<td>-1.0 (-4.8, 2.8)</td>
<td></td>
</tr>
<tr>
<td>Week 4 vs. Baseline</td>
<td>-1.8 (-5.2, 1.5)</td>
<td>-0.9 (-4.2, 2.5)</td>
<td>-0.0 (-4.2, 4.1)</td>
<td></td>
</tr>
<tr>
<td>Week 8 vs. Baseline</td>
<td>-3.4 (-6.3, -0.6)*</td>
<td>-1.8 (-4.7, 1.0)</td>
<td>0.6 (-3.4, 4.7)</td>
<td></td>
</tr>
<tr>
<td><strong>HF-LF Liking Score</strong></td>
<td></td>
<td></td>
<td></td>
<td>0.851</td>
</tr>
<tr>
<td>Baseline</td>
<td>6.1 (6.3)</td>
<td>7.9 (8.8)</td>
<td>1.8 (-1.4, 5.1)</td>
<td></td>
</tr>
<tr>
<td>Week 4 vs. Baseline</td>
<td>-1.6 (-4.7, 1.6)</td>
<td>-2.0 (-5.2, 1.2)</td>
<td>1.4 (-1.7, 4.5)</td>
<td></td>
</tr>
<tr>
<td>Week 8 vs. Baseline</td>
<td>-3.7 (-6.7, -0.6)*</td>
<td>-3.3 (-6.3, -0.3)*</td>
<td>2.2 (-0.3, 4.8)</td>
<td></td>
</tr>
</tbody>
</table>
### Chapter Four: Study 2 – Effect of Dietary Fat Intake and Genetics on Fat Taste Sensitivity: a Co-Twin Randomised Controlled Trial

<table>
<thead>
<tr>
<th></th>
<th>Low-Fat Diet $(n = 44)$</th>
<th>High-Fat Diet $(n = 44)$</th>
<th>Between-Group Differences</th>
<th>Time-Diet Interaction $(P$-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sweet</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.06 (0.36)</td>
<td>1.01 (0.35)</td>
<td>-0.05 (-0.15, 0.06)</td>
<td>0.957</td>
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<tr>
<td>Week 4 vs. Baseline</td>
<td>0.10 (-0.03, 0.23)</td>
<td>0.08 (-0.05, 0.20)</td>
<td>-0.07 (-0.17, 0.04)</td>
<td></td>
</tr>
<tr>
<td>Week 8 vs. Baseline</td>
<td>0.13 (0.00, 0.26)*</td>
<td>0.13 (0.00, 0.26)*</td>
<td>-0.05 (-0.16, 0.06)</td>
<td></td>
</tr>
<tr>
<td><strong>Salty</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.37 (0.35)</td>
<td>1.36 (0.34)</td>
<td>-0.01 (-0.11, 0.09)</td>
<td>0.419</td>
</tr>
<tr>
<td>Week 4 vs. Baseline</td>
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<td>0.01 (-0.11, 0.13)</td>
<td>0.04 (-0.06, 0.14)</td>
<td></td>
</tr>
<tr>
<td>Week 8 vs. Baseline</td>
<td>-0.02 (-0.15, 0.10)</td>
<td>-0.06 (-0.19, 0.06)</td>
<td>-0.05 (-0.15, 0.05)</td>
<td></td>
</tr>
<tr>
<td><strong>Sour</strong></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>1.55 (0.35)</td>
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<td>0.609</td>
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<td>Week 4 vs. Baseline</td>
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<td>0.07 (-0.04, 0.19)</td>
<td>0.02 (-0.08, 0.11)</td>
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<tr>
<td>Week 8 vs. Baseline</td>
<td>0.00 (-0.12, 0.12)</td>
<td>0.00 (-0.12, 0.11)</td>
<td>-0.03 (-0.12, 0.07)</td>
<td></td>
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<tr>
<td><strong>Bitter</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.31 (0.46)</td>
<td>1.25 (0.42)</td>
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<td>0.518</td>
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<tr>
<td>Week 4 vs. Baseline</td>
<td>0.07 (-0.07, 0.21)</td>
<td>0.16 (0.04, 0.29)**</td>
<td>0.03 (-0.09, 0.14)</td>
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<tr>
<td>Week 8 vs. Baseline</td>
<td>0.09 (-0.06, 0.23)</td>
<td>0.14 (0.01, 0.28)*</td>
<td>-0.01 (-0.13, 0.11)</td>
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</tbody>
</table>

*(Table continued on next page)*
<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Week 4 vs. Baseline</th>
<th>Week 8 vs. Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Umami</strong></td>
<td>0.98 (0.38)</td>
<td>-0.06 (-0.21, 0.09)</td>
<td>-0.03 (-0.18, 0.12)</td>
</tr>
<tr>
<td></td>
<td>0.90 (0.38)</td>
<td>-0.03 (-0.18, 0.12)</td>
<td>0.05 (-0.10, 0.20)</td>
</tr>
<tr>
<td></td>
<td>-0.08 (-0.20, 0.05)</td>
<td>-0.05 (-0.17, 0.08)</td>
<td>0.01 (-0.12, 0.13)</td>
</tr>
</tbody>
</table>

Table 4.7 – Low-fat and high-fat diet within- and between-group differences in five basic taste sensitivities over the 8-week trial. Baseline data presented as mean (SD); all differences presented as mean and 95% CI. Between-group differences calculated as high-fat diet – low-fat diet; means, CIs and P-values estimated under a linear mixed model including diet, time, and time-diet interaction as fixed effects; with twin pair as a random effect to account for the correlation between co-twins, and participant as the subject with repeated measures. Post-hoc Sidak’s test and CIs are reported. * P-value < 0.05, ** P-value < 0.01
4.5 Discussion

This study assessed change in FTT after 8 weeks of low-fat and high-fat dietary intake. Following the low-fat diet, FTT rank decreased from 6.8 to 2.6 (approximately 6.1 to 1.8 mM FTT), indicating a large increase in fat taste sensitivity. This is likely due to increased expression of fat taste receptors on lingual taste papillae, as rodent studies have demonstrated that CD36 and FFAR4 expression decreases following high-fat exposure (Gaillard et al., 2008; Laugerette et al., 2005; Martin et al., 2011; Zhang et al., 2011). Following the high-fat diet, FTT rank increased from 6.9 to 8.4 after 8 weeks (approximately 6.3 to 8.7 mM FTT), indicating a decrease in fat taste sensitivity. While the magnitude of the reduction in the high-fat group was much lower than the increase seen in the low-fat group, this is likely because in the high-fat group, total fat intake (g) increased by 23%, whereas in the low-fat group there was a 64% reduction in total fat (g) consumed. Overall, an increase (decrease) in energy from fat by 1% resulted in an increase (decrease) in FTT rank by 0.15, similar to the cross-sectional analysis of these data ($\hat{\beta} = 0.11$) (Costanzo et al., 2017).

Previous intervention studies reported a 1.0% increase in body weight following increased energy from fat (28% to 45%) over 4 weeks (Stewart & Keast, 2012), and a 2.3% reduction in body weight following reduced energy from fat (33% to 16%) over 4 to 6 weeks (Newman et al., 2016; Stewart & Keast, 2012). In the current 8-week trial, 39.0% energy from fat led to a 0.1% body weight increase, and 16.8% energy from fat led to a 1.8% reduction in body weight, which was of lower magnitude than the previous studies, especially considering the longer duration. Importantly, when we assess the change in FTT in the subgroup who maintained body weight, the conclusions were unchanged, suggesting that weight loss is not a factor in altering fat taste sensitivity.
Dietary fat contributed to approximately 33% of energy intake in participants at baseline, similar to the 31-32% energy intake from fat in the Australian adult population (ABS, 2013). Despite efforts to maintain body weight, 11 individuals on the low-fat diet lost more than 2 kg. This demonstrates the difficulty of maintaining weight under a low-fat dietary protocol in free-living individuals, and was likely exacerbated by increasing satiety response to dietary fat. Diets that approach the lower acceptable macronutrient distribution range (AMDR) for fat (NHMRC, 2006) are likely to be useful in Western populations to aid in lowering energy intake and risk of obesity. This is in line with evidence that fat intake is linked to obesity (Hooper et al., 2015) due to being more energy dense than other macronutrients, although it should be cautioned that this association is controversial (Tobias et al., 2015).

Heritability of FTT rank at baseline was calculated to be 8%, which is relatively low compared to heritability of salty (22%) and sour (53%) detection thresholds (Wise et al., 2007). Garneau et al., (2017) reported heritability of 8-19% for linoleic acid intensity ratings at various concentrations, which is similar to the current study. However, intensity rating and detection threshold are different taste dimensions and are not directly comparable. In addition, ICC of MZ and DZ twin pairs were similar. This suggests there is little to no genetic contribution to FTT, but rather familial environment (e.g. diet) is responsible for the concordance within pairs. Also, no significant effect modification of zygosity on the diet was observed, indicating little genetic contribution to diet-mediated change to FTT. It is important to recognise that this does not suggest that genes do not influence FTT at all, as previous studies have found that polymorphisms of CD36 influence fat taste sensitivity (Keller et al., 2012; Pepino et al., 2012). Rather, the current study suggests genetic variation does not make individuals any more or less susceptible to modifying FTT by diet. We hypothesise
that genetic polymorphisms have a role in establishing baseline FTT, while dietary intake modifies it thereon. Although genetic variation has little influence on FTT, there is evidence of genetic contributions to fat preference (Reed, 2010), giving evidence to the contrast between thresholds and hedonics (Druz & Baldwin, 1982).

The expression of fat taste receptors in the oral cavity and throughout the alimentary canal, when activated by fatty acid, initiate the satiety cascade (Keast & Costanzo, 2015, Stewart et al., 2011b). Individuals with impaired fat taste may have lower expression of these receptors (Gaillaird et al., 2008; Laugerette et al., 2005), and therefore have attenuated satiety response following fatty food consumption (Keast et al., 2014; Stewart et al., 2011b). In this way, an individual who has lower sensitivity will feel less full and consume a greater quantity of energy, independent of the hedonic system. Low-fat dieting may aid in increasing expression of fat taste receptors throughout the alimentary canal (Stewart et al., 2011b) leading to increased post-ingestive satiety response to fatty food and reduce passive overconsumption. However, fat taste sensitivity may not be associated with obesity (Tucker et al., 2017). This may be because while impaired fat taste sensitivity does influence increased dietary fat intake, it does not necessarily lead to increased energy intake in all individuals. Furthermore, factors that influence obesity are multifaceted and complex. As demonstrated in rodents (Cartoni et al., 2010), exposure to obesogenic conditions may make fat taste impaired individuals more susceptible to overconsumption of energy and subsequent weight gain.

In other taste modalities, taste thresholds are not associated with hedonics (Druz & Baldwin, 1982) and this was expected to be the same with fat taste. In the current study, there was no significant time-diet interaction for HF-LF liking score, indicating fat taste sensitivity has no influence on fatty food liking. This is in line with a recent
study that did not observe an association between fat taste sensitivity and liking of fatty food (Bolhuis et al., 2018). There is evidence that reduced dietary fat intake decreases preference for fatty foods (Ledikwe et al., 2007; Mattes, 1993). It should be noted that changes in dietary fat intake may affect other sensory acuities. For example, 24-week high-fat dietary intake reduced olfactory sensation in rodents (Thiebaud et al., 2014), which may be a contributor to changed preferences in the previous studies (Ledikwe et al., 2007; Mattes, 1993). However, olfactory acuity was not measured in the current study so we cannot conclude this is the case. Similarly, triglyceride perception was not affected by diet in the current study. This may have been because the concentrations of canola oil used were too low to yield FFA concentrations above detection threshold. A recently published study reported that average canola oil detection thresholds were 11.7 ± 1.8% (Heinze et al., 2017), while the concentrations in the current study ranged from 0-10% which were likely below detection for most participants. While there may be some degree of fatty acid perception in foods containing triglycerides due to FFA (Heinze et al., 2017; Koriyama et al., 2002) and lingual lipase activity (Kulkarni & Mattes, 2014; Pepino et al., 2012), this does not have a noticeable impact on preference in most cases. Foods that contain high amounts of FFA (e.g. some oils and nuts) may be more noticeable to individuals who are more sensitive to fatty acids. However, there was no sign of time-diet interaction for liking of the test foods.

This randomised controlled trial has limitations that should be noted when interpreting these results. First, since the trial could not be blinded, we cannot rule out some level of contamination with co-twins mutually discussing their diets. This type of contamination is expected to bias the estimated diet effect toward the null; therefore, the true effect of the diet on FTT should be even larger than the one reported. Second,
while the number of twin pairs was adequate to assess the dietary intervention and heritability, the small number of DZ twin pairs recruited is not powered to detect small differences in effect modification of zygosity on the diet, and these estimates should be interpreted as only indicative of a minimal impact of genetic variation on FTT. Third, the use of one 24-hour dietary recall as a baseline measure of dietary intake only provided a snapshot of a participant’s usual diet. In addition, diet recalls and records are subject to bias and under-reporting in many studies, especially in obese individuals, and we cannot rule out bias and under-reporting in this study. Fourth, while there was no effect of diet on intensity ratings to the five prototypical tastants, it is acknowledged that intensity ratings are not directly comparable to FTT. Finally, while satiety can be inferred based on findings from Stewart et al. (2011b), we did not measure satiety ratings so we cannot conclude whether this trial had an influence on satiety.

4.6 Conclusion

The current study demonstrates that 8 weeks on a low-fat diet increases sensitivity to fat taste, whilst the same period on a high-fat diet attenuates sensitivity, regardless of body weight. There is little indication of genetic contribution on fat taste. Therefore, dietary fat intake is the most important influencer on fat taste sensitivity. Diets that approach the lower AMDR for fat may aid in increasing satiety response to fatty food, decrease passive overconsumption, and subsequently reduce body weight.
Chapter Five: Study 3 – Effect of Dietary Fat Intake on Fat Taste Receptor Gene Expression: a Co-Twin Randomised Controlled Trial

5.1 Introduction

Sensitivity to fat taste is involved in the regulation of dietary fat intake, where individuals with impaired fat taste sensitivity are more likely to consume greater amounts of dietary fat (Costanzo et al., 2017; Keast et al., 2014; Kindleysides et al., 2017; Stewart et al., 2010; 2011a; Tucker et al., 2014). This is due to reduced cephalic phase and post-prandial satiety hormone responses following oral fat exposure (Keast et al., 2014; Little & Feinle-Bisset, 2011; Smeets & Westerterp-Pantenga, 2006, Stewart et al., 2011b; Wisén et al., 1992). This is reflected throughout the alimentary canal, as individuals who are less sensitive to fat taste in the oral cavity also have reduced hormonal response following fatty acid stimulation in the GIT (Stewart et al., 2011b). Measures of fat taste sensitivity can therefore be useful proxy for dietary fat intake, digestion, and subsequent post-digestive responses.

Fat taste sensitivity is attenuated by oral fat exposure, and conversely can be increased by long-term reduced dietary fat intake (Costanzo et al., 2018; Newman et al., 2016; Stewart & Keast, 2012) which is likely due to regulation of fat taste receptors in the oral cavity (Figure 5.1). In rodents, oral exposure to dietary fat downregulates the levels CD36 and FFAR4 in taste papillae (Martin et al., 2011; Zhang et al., 2011), however this has not been investigated in humans. Three lingual papillae – fungiform,
 folate and circumvallate – house taste bud cells which express the following fat taste receptors in humans: CD36, FFAR2, FFAR4, GPR84 and KCNA2 (Galindo et al., 2012; Liu et al., 2018; Simons et al., 2011). Receptors have specificities to different chain lengths and level of saturation of fatty acid, although there is some crossover (Liu et al., 2016). CD36 and FFAR4 are involved in the detection of LCFA; FFAR4 and GPR84 are involved in the detection of MCFA; FFAR2 and GPR84 are involved in the detection of SCFA; and KCNA2 is involved in the detection of PUFA. However, there is some evidence for coordination between receptors via signal transduction cascades rather than functioning independently. LCFAs bind to CD36 which then triggers an intracellular signal transduction to communicate with FFAR4 (Abdoul-Azize et al., 2014). This is supported by the co-expression of CD36 and FFAR4 on single TBCs (Ozdener et al., 2014), and the higher affinity of CD36 for LCFA compared to FFAR4 (Silverstein & Febbraio, 2009; Smith, 2012).

It should be noted that SCFA may have a taste quality independent of the current concept of ‘fat taste’, and may be more akin to sour taste (Running et al., 2015), potentially acting as a warning system against ingestion of spoiled food. It is unlikely that dietary fat will mediate FFAR2 and GPR84, as naturally occurring SCFA is uncommon in food. Instead, these receptors are greatly expressed in the GIT, and likely have a more important role in nutrient-sensing of SCFA produced from dietary fibre digestion by GIT biota (Kles & Chang, 2006).

Understanding factors causal to fat taste function and receptor regulation is important, as potential therapies may be formulated to simulate oral fat exposure and increase satiety responses. This may lead to reduced consumption of fatty foods, and therefore a reduction in energy intake and obesity risk.
Chapter Five: Study 3 – Effect of Dietary Fat Intake on Fat Taste Receptor Expression: a Co-Twin Randomised Controlled Trial

Figure 5.1 – Schematic representation of fat taste sensitivity and receptor gene expression regulation following long-term high-fat (left) and low-fat (right) dietary intake. Original diagram. (1) Dietary fat is predominately in the form of triglyceride (TG), a small portion of which is broken down to free fatty acid (FFA) via lingual lipase activity in the oral cavity. Additionally, small amounts of FFA can occur naturally in food. (2) FFA enters taste buds and activates fat taste receptors (CD36, FFAR2, FFAR4, GPR84, and KCNA2) embedded on taste bud cell membranes. Long-term low-fat intake increases gene expression of FFAR4 and KCNA2 (specific to polyunsaturated fatty acid intake), and causes heightened fat taste sensitivity following fatty acid exposure; while the opposite is true for long-term high-fat intake. A fat taste signal leads to a range of physiological responses including plasma TG mobilisation, transient stimulation of cholecystokinin (CCK), pancreatic polypeptide (PP) and peptide YY (PYY), gastric lipase secretion, and satiety. It is speculated that a greater fat taste signal will result in stronger physiological responses. (3) Following dietary fat ingestion, gastric and pancreatic lipase plays a further role in the liberation of FFA from TG, which then activate fatty acid receptors on enteroendocrine cells, stimulating secretion of satiety hormones CCK, PYY and glucose-like peptide-1 (GLP-1), where individuals with heightened fat taste sensitivity secrete greater levels of satiety hormones. (4) Following the satiety responses, individuals with heightened fat taste sensitivity reach satiety earlier, and subsequently consume less fat and energy, than individuals with impaired fat taste sensitivity.
5.2 Aims & Hypotheses

5.2.1 Aims
The aim of this study was to assess the effect of low-fat (<20% energy from fat) or high-fat (>35% energy from fat) dietary intake on fasting expression of fat taste receptor genes (CD36, FFAR2, FFAR4, GPR84 and KCNA2) in fungiform papillae.
This study also aims to assess the associations between fat taste receptor genes and C18:1 FTT. A secondary aim was to explore if nutrient intakes other than fat had an influence on fat taste receptor gene expression. A co-twin design was chosen as it controls for age, and genetic and common environmental factors shared by co-twins in each experimental group.

5.2.2 Hypotheses
- Low-fat dietary intake will increase expression of fat taste receptor genes, particularly those involved in LCFA and MCFA detection (CD36, FFAR4, and KCNA2).
- High-fat dietary intake will decrease expression of fat taste receptor genes, particularly those involved in LCFA and MCFA detection (CD36, FFAR4, and KCNA2).
- There will be positive associations between C18:1 FTT and expression of LCFA receptor genes (CD36 and FFAR4).
- PUFA intake will be associated with KCNA2 expression.
Chapter Five: Study 3 – Effect of Dietary Fat Intake on Fat Taste Receptor Expression: a Co-Twin Randomised Controlled Trial

5.3 Participants, Materials, and Methods

5.3.1 Participants

Twins were eligible to participate in the study if they were aged between 18-69 years, were able to attend three laboratory sessions in Burwood, VIC, and were willing to alter their diet for a period of 8 weeks. Both MZ and DZ twin pairs were invited to participate. Subjects were excluded from recruitment if they had any dairy allergies and intolerances, illnesses preventing them from eating foods included in the study, or if they were pregnant or lactating. Due to the nature of the twin study design, if one individual from a twin pair was excluded or withdrew from the study, their co-twin was also excluded.

Sixty-six twin pairs (132 individuals) were screened by TRA to participate in this study, as described in Chapter 2.2.1. Forty-six twin pairs (92 individuals) aged between 18-68 years were recruited into Study 2, and of that sample, 13 pairs (26 individuals) consented to additionally participate in this study. Co-twins from each pair were randomised into either a low-fat or high-fat diet. Prior to recruitment, a block randomised sequence was generated in sizes of two. TRA was responsible for recruitment and therefore characteristics of the participants were blinded to the researchers. Participants were allocated to the randomised sequence based on their TRA twin number; therefore, allocation of participants to diet group was concealed. Due to the nature of the intervention, blinding of participants was not feasible. Ethics was approved by the Deakin University Human Research Ethics Committee (2013-163) in accordance with the Declaration of Helsinki, and informed written consent was obtained by all participants prior to participation. This study was registered at www.anzctr.org.au as a clinical trial (ID: ACTRN12613000466741).
5.3.2 Study Outline

Participants attended two tasting sessions and two biopsy sessions at the Centre for Advanced Sensory Science at Deakin University, Burwood, VIC. The first tasting session occurred on the day prior to beginning the intervention, and the second tasting session occurred on the last day of the intervention. Tasting tests were conducted in a temperature and sound controlled environment with a 15 minute break in the middle of their session to prevent fatigue. Participants were asked to avoid eating or drinking anything but water and to avoid brushing their teeth or using mouthwash up to an hour prior to each tasting session. Tasting sessions measured C18:1 FTT and anthropometric measurements. A 24-hour food recall was collected by a nutritionist during the first session. Between tasting sessions, participants recorded three 24-hour diet records (2 weekdays, 1 weekend day) to determine dietary compliance.

Each biopsy session occurred on the morning following a tasting session. Participants fasted for at least 10 hours prior to each biopsy session. Biopsy sessions collected fungiform papillae tissue and blood serum samples. After the biopsy session, participants were provided with a low-fat or high-fat breakfast snack depending on which diet they were allocated.

5.3.3 Dietary Intervention

The low-fat diet was defined as <20% of energy from fats and the high-fat diet was defined as >35% of energy from fats, as described in Chapter 2.7. Participants were requested to start the assigned diet the day after baseline measurement. As foods were not provided in this study, food choice was up to the participants. To maximise adherence to the diets, participants were contacted via phone fortnightly and
questioned on their dietary habits, described in detail in **Chapter 2.7**. Food records, as described in **Chapter 2.8.2**, were inspected at the beginning of sessions 2 and 3, and reviewed for adherence to the assigned diet.

### 5.3.4 Dietary Assessment

A single three-pass 24-hour dietary recall of the day prior to session 1 was conducted by a trained nutritionist to assess short-term dietary intake, as described in **Chapter 2.8.1**. Participants also completed three 24-hour diet records between session 1 & 2; and between session 2 & 3 (Figure 4.1), as described in **Chapter 2.8.2**.

### 5.3.5 Anthropometry

Body weight, height, BMI, waist circumference and hip circumference were measured as described in **Chapter 2.6**.

### 5.3.6 Fat Taste Receptor Gene Expression

Fungiform papillae biopsy was conducted without anaesthetic by a registered doctor as described in **Chapter 2.5**. For each participant, up to 8 fungiform papillae were collected and pooled as an individual sample. Samples were sent to CSIRO for analysis of fat taste receptor gene expression, conducted by researchers independent to this PhD. The expression of fat taste receptor genes including *CD36*, *FFAR2*, *FFAR4*, *GPR84* and *KCNA2* were analysed with real time RT-PCR. RNA was extracted from the pooled fungiform papillae samples The RNA integrity was measured with Bioanalyser 2100. For the RT-PCR, 1μg of total RNA was used to
5.3.7 Fat Taste Threshold

FTT was measured using established methods (Haryono et al., 2014), as described in Chapter 2.4.1. An ascending series 3-AFC methodology was used, where participants were provided with multiple sample sets each containing three randomly-ordered samples of fat-free milk per set, two controls and one containing C18:1 at varying concentrations (0.02, 0.06, 1.00, 1.40, 2.00, 2.80, 3.80, 5.00, 6.40, 8.00, 9.80, 12.00, and 20.00 mM). Participants were asked to taste each sample in the set and identify the sample that matched the taste quality from the warm-up sets, described further in Chapter 2.4.1. Correct identification of the C18:1 sample resulted in the participants repeating the same sample set. Incorrect identification of the C18:1 sample resulted in a new sample set with a higher concentration of C18:1. FTT was defined as the concentration of C18:1 correctly identified in three consecutive sample sets of the same concentration. FTT was transformed to an ordinal variable — FTT rank — ranging from 0 to 12, with higher ranks implying lower sensitivity to fat taste (Table 2.1).

5.3.8 Statistical Analysis

Analyses were conducted using computer software SPSS. Null hypotheses were rejected at $P < 0.05$. The effect of the diet on fat taste receptor gene expressions was assessed using linear mixed models including diet group (low-fat and high-fat), time (baseline and week 8) and the interaction diet by time as fixed effects; with twin pair synthesise cDNA. The expression of the interested genes were analysed with the Taqman gene expression assays (Table 2.2).
as a random effect and co-twin as the subject with repeated measures to account for
the correlation between co-twins. Time-diet interaction, post-hoc Sidak’s test and
confidence intervals are reported.

To explore the strength of the association (\( \beta \)) between change in FTT rank and change
in fat taste receptor gene expression over the 8 week period, a linear mixed model was
used with \( \Delta \) FTT rank (\( \Delta \), week 8 – baseline) as the outcome and \( \Delta \) gene expression
as a fixed effect; with twin pair as a random effect. The Pearson’s correlation (\( r \))
between \( \Delta \) FTT rank and \( \Delta \) gene expression is also reported for descriptive purposes.
The same analysis was repeated for the associations between change in intake of
nutrients (total fat, saturated fat, monounsaturated fat, polyunsaturated fat,
carbohydrate, protein, and dietary fibre) (g) and \( \Delta \) gene expression to see if nutrients
other than fat affect gene expression.

5.4 Results

All 13 twin pairs (10 MZ, 3 DZ; 9 female pairs, 4 male pairs) completed this study.
However, 4 twin pairs were not included in the RT-PCR analysis due to low quantity
or poor integrity of the collected RNA samples according to the Nanodrop and
Bioanalyser analyses. As a result, a total of 36 samples were analysed (9 pairs at both
baseline and week 8). We present here the 18 individuals (9 pairs) with valid samples
that underwent RT-PCR analysis. Baseline characteristics of the participants are
described in Table 5.1.
## Chapter Five: Study 3 – Effect of Dietary Fat Intake on Fat Taste Receptor Expression: a Co-Twin Randomised Controlled Trial

### 5.4.1 Fat Taste Threshold

Compared with baseline, the low-fat group FTT rank significantly decreased from 8.1 to 2.3 (-5.8 [-8.1, -3.5], $P < 0.001$) at week 8 and the high-fat group FTT rank significantly increased from 7.0 to 9.3 (+2.3 [0.1, 4.6], $P = 0.042$) at week 8. There was a significant between-group difference of 7.0 [4.2, 9.8] at week 8, and there was a significant time-diet interaction for FTT rank ($P < 0.001$).

### 5.4.2 Fat Taste Receptor Expression

There was a weak time-diet interaction for $FFAR4$ expression ($P = 0.063$), as expression significantly increased on the low-fat diet ($P = 0.023$) (Table 5.2). No significant time-diet interactions were observed for $CD36$, $GPR84$, $FFAR2$ and $KCNA2$ expression, although there was a significant difference in $GPR84$ expression between diet groups at baseline.
### Table 5.2 – Within- and between-group mean differences in fasting expression of fat taste receptor genes over the 8-week trial

Data presented as mean and 95% CI. Between-group difference calculated as high-fat diet – low-fat diet; Means, CIs and P-values estimated under a linear mixed model including diet, time, and time-diet interaction as fixed effects; with twin pair as a random effect to account for the correlation between co-twins, and participant as the subject with repeated measures. Post-hoc Sidak’s test, CI and time-diet interaction are reported. *P < 0.05

<table>
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<th>Gene</th>
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<th>High-Fat Diet (n = 9)</th>
<th>Between-Group Difference</th>
<th>Time-Diet Interaction (P-value)</th>
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<tr>
<td>CD36</td>
<td>1.20 (0.65, 1.75)</td>
<td>1.26 (0.71, 1.81)</td>
<td>0.06 (-0.56, 0.68)</td>
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<tr>
<td>FFAR4</td>
<td>0.84 (0.57, 1.12)</td>
<td>0.93 (0.66, 1.21)</td>
<td>0.09 (-0.30, 0.48)</td>
<td>0.063</td>
</tr>
<tr>
<td>FFAR2</td>
<td>0.95 (0.53, 1.36)</td>
<td>0.60 (0.18, 1.01)</td>
<td>-0.35 (-0.93, 0.23)</td>
<td>0.409</td>
</tr>
<tr>
<td>GPR84</td>
<td>0.057 (0.020, 0.095)</td>
<td>0.022 (-0.015, 0.060)</td>
<td>-0.035 (-0.069, -0.001)*</td>
<td>0.214</td>
</tr>
<tr>
<td>KCNA2</td>
<td>0.17 (0.12, 0.22)</td>
<td>0.18 (0.14, 0.23)</td>
<td>0.01 (-0.04, 0.07)</td>
<td>0.460</td>
</tr>
</tbody>
</table>

*P < 0.05
5.4.3 Associations between Fat Taste Threshold and Fat Taste Receptor Gene Expressions

The relationship between fat taste sensitivity and fat taste receptor gene expression was assessed by comparing change in FTT rank with change in gene expression from baseline to week 8 (Table 5.3 & Figure 5.1). There was a significant negative association between Δ FTT rank and Δ \( \text{FFAR4} \), indicating that as \( \text{FFAR4} \) expression increased, FTT rank decreased. Conversely, the positive association between Δ FTT rank and Δ \( \text{GPR84} \) indicates that as \( \text{GPR84} \) expression increased, FTT rank also increased. There were no associations between Δ FTT rank and Δ \( \text{CD36} \), Δ \( \text{FFAR2} \) and Δ \( \text{KCNA2} \).

<table>
<thead>
<tr>
<th>ΔFTT rank</th>
<th>( r )</th>
<th>( \hat{\beta} )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ ( \text{CD36} )</td>
<td>-0.061</td>
<td>-0.8</td>
<td>0.822</td>
</tr>
<tr>
<td>Δ ( \text{FFAR4} )</td>
<td>-0.590</td>
<td>-8.8</td>
<td>0.016</td>
</tr>
<tr>
<td>Δ ( \text{FFAR2} )</td>
<td>0.270</td>
<td>3.4</td>
<td>0.311</td>
</tr>
<tr>
<td>Δ ( \text{GPR84} )</td>
<td>0.517</td>
<td>68.7</td>
<td>0.040</td>
</tr>
<tr>
<td>Δ ( \text{KCNA2} )</td>
<td>-0.302</td>
<td>-21.3</td>
<td>0.256</td>
</tr>
</tbody>
</table>

Table 5.3 – Associations between Δ FTT rank and change in fat taste receptor gene expression. Δ, week 8 – baseline; \( \hat{\beta} \) and \( P \)-values obtained under a linear mixed model including twin pair as a random effect; \( r \) obtained using Pearson’s correlation for descriptive purposes.
Figure 5.2 – Scatterplots of Δ FTT rank vs. change in fat taste receptor gene expression. Circle markers (○) indicate participants in the low-fat group (n = 9); square markers (□) indicate participants in the high-fat group (n = 9). Figure A: Δ FTT rank vs. Δ CD36; Figure B: Δ FTT rank vs. Δ FFAR4; Figure C: Δ FTT rank vs. Δ FFAR2; Figure D: Δ FTT rank vs. Δ GPR84; Figure E, Δ FTT rank vs. Δ KNCA2.
5.4.4 Associations between Nutrient Intakes and Fat Taste Receptor Gene Expressions

There were significant negative associations between ΔFFAR4 and Δ fat intake (g) ($\hat{\beta} = -159.4; r = -0.744; P < 0.001$), Δ saturated fat intake (g) ($\hat{\beta} = -79.4; r = -0.759; P < 0.001$), Δ monounsaturated fat intake (g) ($\hat{\beta} = -53.4; r = -0.711; P = 0.001$), and Δ polyunsaturated fat intake (g) ($\hat{\beta} = -14.8; r = -0.533; P = 0.023$). There was weak evidence for a negative association between ΔKCNA2 and Δ polyunsaturated fat intake ($\hat{\beta} = -72.7; r = -0.459; P = 0.056$). Finally, there was a significant positive association between ΔFFAR2 and Δ dietary fibre intake ($\hat{\beta} = 22.5; r = 0.560; P = 0.016$). No significant associations were observed for ΔCD36 or ΔGPR84, although there was a significant association between baseline GPR84 expression and intake of energy from dietary fibre (%) ($\hat{\beta} = 8.8; r = 0.399; P = 0.023$).

5.4.5 Dietary Compliance

Compared with baseline, there was a significant reduction in energy from fat in the low-fat group after 8 weeks ($P < 0.001$). Intake of energy from fat did not increase significantly in the high-fat group after 8 weeks. Week 8 intakes of energy from fat were within the aims of the trial, with the low-fat group consuming 14.8 (6.5) % energy from fat and the high-fat group consuming 39.9 (4.4) % energy from fat.

There were significant time-diet interactions for intakes of energy (kJ: $P = 0.016$), total fat (g: $P = 0.006$; % energy: $P < 0.001$), saturated fat (g: $P = 0.030$; % energy: $P = 0.002$), monounsaturated fat (g: $P = 0.001$; % energy: $P < 0.001$), polyunsaturated fat (g: $P = 0.004$; % energy: $P = 0.004$), protein (% energy: $P = 0.010$), and carbohydrate (% energy: $P = 0.003$) (Tables 5.4 & 5.5).
### Table 5.4 – Low-fat and high-fat diet within- and between-group mean differences in dietary fat intake in Study 3.

Baseline data presented as mean (SD); all differences presented as mean and 95% CI. Between-group differences calculated as high-fat diet – low-fat diet; Means, CIs and P-values estimated under a linear mixed model including diet, time, and time-diet interaction as fixed effects; with twin pair as a random effect to account for the correlation between co-twins, and participant as the subject with repeated measures. Post-hoc Sidak’s test and CIs are reported. * P-value < 0.05; ** P-value < 0.01; *** P-value < 0.001; Mono, Monounsaturated; Poly, Polyunsaturated.

<table>
<thead>
<tr>
<th></th>
<th>Low-Fat Diet (n = 9)</th>
<th>High-Fat Diet (n = 9)</th>
<th>Between-Group Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g</td>
<td>% Energy</td>
<td>g</td>
</tr>
<tr>
<td>Total Fat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>107.4 (92.9)</td>
<td>35.1 (7.8)</td>
<td>83.3 (39.6)</td>
</tr>
<tr>
<td>Week 8 vs. Baseline</td>
<td>-77.6 (-127.4, -27.8)**</td>
<td>-20.3 (-26.2, -14.4)***</td>
<td>28.4 (-21.4, 78.2)</td>
</tr>
<tr>
<td>Saturated Fat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>45.5 (49.2)</td>
<td>13.7 (4.2)</td>
<td>34.8 (21.0)</td>
</tr>
<tr>
<td>Week 8 vs. Baseline</td>
<td>-37.2 (-63.4, -10.9)**</td>
<td>-9.5 (-13.2, -5.8)***</td>
<td>4.6 (-21.7, 30.9)</td>
</tr>
<tr>
<td>Mono Fat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>39.3 (28.7)</td>
<td>13.1 (3.5)</td>
<td>30.5 (12.5)</td>
</tr>
<tr>
<td>Week 8 vs. Baseline</td>
<td>-28.1 (-44.0, -12.2)**</td>
<td>-7.7 (-9.9, -5.4)***</td>
<td>13.7 (-2.2, 29.6)*</td>
</tr>
<tr>
<td>Poly Fat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>13.2 (8.9)</td>
<td>4.6 (1.8)</td>
<td>11.7 (6.5)</td>
</tr>
<tr>
<td>Week 8 vs. Baseline</td>
<td>-6.9 (-13.2, -0.6)*</td>
<td>-1.6 (-2.8, -0.0)*</td>
<td>7.3 (1.1, 13.6)*</td>
</tr>
</tbody>
</table>
Table 5.5 – Low-fat and high-fat diet within- and between-group mean differences in energy, protein, carbohydrate, alcohol, and fibre intake in Study 3. Baseline data presented as mean (SD); all differences presented as mean and 95% CI. Between-group differences calculated as high-fat diet – low-fat diet; Means, CIs and P-values estimated under a linear mixed model including diet, time, and time-diet interaction as fixed effects; with twin pair as a random effect to account for the correlation between co-twins, and participant as the subject with repeated measures. Post-hoc Sidak’s test and CIs are reported. * P-value <0.05; ** P-value <0.01; *** P-value <0.001
5.5 Discussion

This study assessed changes in fasting expression of fat taste receptor genes following 8 weeks of low-fat or high-fat dietary intake. It is well established that fat taste sensitivity is modulated by dietary fat intake (Costanzo et al., 2018; Newman et al., 2016; Stewart & Keast 2012). It was hypothesised that expression of fat taste receptors would be similarly modulated due to dietary fat intake, as in rodent models (Martin et al., 2011; Zhang et al., 2011). However, only \textit{FFAR4} was affected by the diet, although this did not reach significance ($P = 0.063$). Fasting expression of \textit{FFAR4} increased by 38% in the low-fat diet group from baseline to week 8. While the magnitude of reduction in \textit{FFAR4} expression in the high-fat group (3%) was much lower than the increase seen in the low-fat group, this is likely because in the high-fat group, fat (g) increased by only 34% whereas in the low fat group there was a 72% reduction in fat consumed. Further, change in \textit{FFAR4} expression was associated with change in C18:1 taste thresholds, or in other words as expression increased, fat taste sensitivity also increased. These results are also supported by the associations between \textit{FFAR4} expression and intakes of fat, saturated fat, monounsaturated fat and polyunsaturated fat, indicating that fatty acids with any level of saturation may downregulate \textit{FFAR4} expression. \textit{FFAR4} expression is known to have a role in fat taste function and preference for fatty foods (Cartoni et al., 2010; Martin et al., 2011), but this is the first time the expression of \textit{FFAR4} and its association with taste function has been reported in humans.

Interestingly, there was no significant effect of the diet on \textit{CD36} expression in the current study. There was a small trend in the same direction as \textit{FFAR4}, in that expression of \textit{CD36} increased by 16% in the low-fat diet and decreased by 13% in the high-fat diet after 8 weeks. Short-term oral exposure to dietary fat in mice decreased
Chapter Five: Study 3 – Effect of Dietary Fat Intake on Fat Taste Receptor Expression: a Co-Twin Randomised Controlled Trial

CD36 levels by two-fold within 1 hour of refeeding (Martin et al., 2011). However, 11 hours of fasting returned CD36 levels to pre-prandial levels. Therefore, we speculate that the role of CD36 is to mediate short-term response to dietary fat, while FFAR4 is involved in regulating long-term response to dietary fat. Analysis of CD36 expression in human papillae immediately before and after consumption of dietary fat is necessary to confirm this.

While the dietary intervention was not designed to control for polyunsaturated fat intake as participants were able to choose to consume any food sources of fat, intake of polyunsaturated fat increased on the low-fat diet and decreased on the high-fat diet. Despite this, time-diet interaction for KCNA2 expression was not significant, which opposes our hypothesis as KNCA2 encodes the receptor responsible for PUFA detection (Liu et al., 2016). However, there was an 18% increase in KCNA2 expression observed in the low-fat diet group which was in the hypothesised direction, and essentially no change to KCNA2 expression in the high-fat diet group likely due to the minor increase in fat intake over the 8 weeks. Furthermore, there was weak evidence for an association between Δ KCNA2 and Δ polyunsaturated fat intake (\( P = 0.056 \)), which indicates there is some effect of polyunsaturated fat intake on KCNA2 expression.

It was expected that there would be no effect of the diet on FFAR2 and GPR84, as these receptors are mainly responsible for SCFA detection (Liu et al., 2016). There is little SCFA in dietary fat and, although not measured, we believe there would not have been a large difference in SCFA intake at week 8 compared with baseline. However, we did observe a significant association between Δ FFAR2 and Δ dietary fibre intake (\( P = 0.016 \)). While this was not the intent of the intervention, dietary fibre intake was not a controlled variable and therefore was subject to incidental changes in both diet
groups. Additionally, there was an association between GPR84 and energy from dietary fibre (%) \( (P = 0.023) \). These are interesting associations, as increased dietary fibre intake leads to increased production of SCFA by biota in the GIT (Kles & Chang, 2006). Since regulation of taste receptors is presumably analogous throughout the alimentary canal (Stewart et al., 2011b), it is possible that increased exposure to SCFA in the GIT may cause of the increased expression of lingual FFAR2. However, this cannot be concluded in the current study.

Although the results seem to indicate minor changes to gene expression, it should be noted that these changes were, for the most part, in the hypothesised directions, particularly CD36, FFAR4 and KCNA2. Furthermore, there was a strong time-diet interaction for FTT \( (P < 0.001) \), suggesting that there must be some physiological change to taste mechanisms that is causing the change in FTT. However, the taste system is multidimensional and complex, and there are a number of reasons why the changes in gene expression were small despite a large change in FTT. First, while not statistically significant, small changes to gene expression may be sufficient to cause large changes in FTT. To that point, we only measured gene expression in fungiform papillae whereas there may have been much larger changes to gene expression in foliate and circumvallate papillae. For example, levels of CD36 are greater in foliate and circumvallate papillae than fungiform in rodent TBCs (Gilbertson et al., 2005). Second, coordination and interaction of receptors is complex and may confound the results. For example, upregulation of either FFAR4 or CD36 independently may have an insignificant effect on FTT, but when upregulated together there may be a larger attenuation of FTT due to intracellular signal transduction (Abdoul-Azize et al., 2014). Finally, there may be salivary factors that were not measured in this study that are causal to the large FTT change. For example, antioxidant capacity, protein content and
lysozyme activity in saliva have been shown to be associated with FTT (Mounayar et al., 2013; Poette et al., 2014).

This randomised controlled trial has some limitations that should be noted when interpreting these results. First, the analysis of fat taste receptor gene expressions was only conducted in fungiform papillae, as collection of foliate and circumvallate papillae in living humans is difficult. The relative expressions of these genes within and between human taste papillae are not known, so therefore the results from this study should be interpreted only as indicative of the entire oral cavity. Second, the sample size was small so there may not have been enough power to detect small changes in gene expression. However, the sample size had sufficient power to detect changes in FTT. Third, while we explored associations between nutrient intakes and fat taste receptor gene expressions, this study was designed as an intervention to dietary fat intake. Changes in other nutrient intakes were incidental, and therefore the observed associations need to be confirmed in trials that are designed around those nutrients specifically. Lastly, due to the small sample of twin pairs, quantitative genetic effects could not be evaluated in this study.

5.6 Conclusion

The current study demonstrates that 8 weeks on a low-fat diet increases expression of $FFAR4$, which is likely the cause in changes to FTT following dietary fat mediation. In addition, the results indicate that $FFAR4$ is responsible for regulating long-term satiety and desire to consume fatty foods. $KCNA2$ may also have some role in regulating intake of polyunsaturated fat although more research is needed.
Chapter 6: Study 4 – Regional Sensitivities to Fatty Acid on the Human Tongue

6.1 Introduction

Fat taste is initiated by the chemoreception of fatty acid by putative fat taste receptors on TBCs housed within structures known as papillae in the oral cavity (Keast & Costanzo, 2015). Taste papillae are located at regional clusters on the tongue: fungiform (anterior tongue), foliate (posterior lateral tongue), circumvallate (posterior medial tongue) (Figure 1.1). TBCs within taste papillae express receptors responsible for the basic taste modalities. For example, GPR families T1R (sweet and umami) and T2R (bitter) are expressed in type II TBCs embedded mainly within circumvallate and foliate papillae (Nelson et al., 2001), with reduced expression of T2Rs also identified within fungiform TBCs (Adler et al., 2000). While the ‘tongue map’ concept has been discarded, there are differences in taste sensitivities between loci of the tongue. For example, sweet taste is more intense at the tip of the tongue (fungiform region) compared to the base of the tongue (circumvallate region) (Sato et al., 2009). This has not been explored for fat taste, although it is reasonable to expect that there are differences in fat taste sensitivity between loci as well.

Both animal and human studies have shown a number of receptors to be involved in fat taste function, including CD36, FFAR1, FFAR2, FFAR3, FFAR4, GPR84 and KCNA2 (Liu et al., 2018; Simons et al., 2011; Zhang et al., 2011). CD36 has been shown to be expressed in human circumvallate, foliate and fungiform TBCs (Liu et
Chapter Six: Study 4 – Regional Sensitivities to Fatty Acid on the Human Tongue

al., 2018; Ozdener et al., 2014; Simons et al., 2011). Relative ratios of CD36 within different papillae TBCs has not been investigated in humans, although there is a lower expression of CD36 within fungiform TBCs in rodents (Gilbertson et al., 2005). FFAR4 has only been reported in type II TBCs within circumvallate and fungiform papillae in humans (Galindo et al., 2011), while expression has additionally been reported within mouse foliate TBCs (Cartoni et al., 2010). FFAR2, GPR84 and KCNA2 have recently been identified in human fungiform TBCs (Liu et al., 2018).

It is important to understand the functional properties of fat taste receptors, as fat taste sensitivity has been shown to be linked with dietary intake. For example, impaired fat taste sensitivity is associated with increased dietary fat consumption (Costanzo et al., 2017; Newman et al., 2016; Stewart et al., 2010; Stewart & Keast, 2012; Tucker et al., 2015), and reduced satiety response to fatty foods (Bolhuis et al., 2015; Keast et al., 2014; Stewart et al., 2011). Accurate measurement of fat taste sensitivity in humans is important to understand fat taste function, and may aid in development of strategies to reduce consumption of fatty foods and therefore obesity. Currently, there is no agreement on the best method for measuring fat taste sensitivity (Heinze et al., 2015). Such methods include the triangle test (Stewart et al., 2011), 3-alternative forced choice (3-AFC) method (Haryono et al., 2014), 2-AFC method (Mattes, 2009) and the staircase method (Tucker et al., 2014), where Heinze et al., (2015) has suggested the 3-AFC method to be the gold standard for measuring fat taste sensitivity. Further, liquid vehicles are primarily used for these methods: skim milk (Haryono et al., 2014) or water (Tucker & Mattes, 2013). However, all of these methods are lengthy to prepare and conduct particularly outside of a laboratory setting, require repeated measures to reduce false positives, and can cause sensory fatigue at high concentrations. Liquid vehicles do not discriminate between tongue loci. It is not
known whether different papillae clusters have different taste sensitivities or some papillae clusters are more susceptible to fatty acid stimulation. Understanding the relative taste function of different papillae clusters may aid in the development of novel rapid fat taste threshold methods.

### 6.2 Aims & Hypotheses

#### 6.2.1 Aims

This study aimed to identify the regions of the tongue most susceptible to stimulation of C18:1 using a cotton bud. It also aims to compare FTT throughout the entire oral cavity using a liquid vehicle (FTT-OC) and FTT in fungiform papillae clusters using filter paper strips (FTT-F).

#### 6.2.2 Hypotheses

- Circumvallate and foliate papillae will be more responsive to fatty acid stimulation compared with fungiform papillae due to higher expression of CD36 in rodent models.
- FTT-OC will be correlated with FTT-F.

### 6.3 Participants, Materials, and Methodology

#### 6.3.1 Study Outline

Participants attended three sessions, approximately one week apart, at the Centre for Advanced Sensory Science at Deakin University, Burwood, VIC. In the first session,
Chapter Six: Study 4 – Regional Sensitivities to Fatty Acid on the Human Tongue

A papillae stimulus test was conducted to assess the relative susceptibility of each papillae location of the tongue. FTT was measured during the second and third sessions, where a liquid or solid vehicle was used for either session in a random stratified order (for example, 50% of participants completed the solid vehicle test in the second session and liquid vehicle in the third session). All tests were conducted in temperature and sound controlled partitioned sensory booths. Participants wore nose clips during testing to prevent odour cues and red lights were used to reduce visual cues. Participants also rinsed their mouth with deionized water before tasting each sample or sample set. Participants were asked to avoid eating or drinking anything but water and to avoid brushing their teeth or using mouthwash up to an hour prior to all laboratory sessions.

6.3.2 Participants

Participants were recruited by email and flyers around Deakin University, Burwood, VIC, as described in Chapter 2.2.1. Only individuals with healthy BMI (18.5-25.0 kg/m$^2$) aged between 18 to 50 years were eligible to participate in either of the studies. Twenty individuals (age range: 18 to 42 years) participated in this study. All twenty participants completed the first session, however only sixteen participants returned for the second and third sessions. The study was conducted in accordance with the Declaration of Helsinki, the protocol was approved by the Deakin University Human Research Ethics Committee (2014-122), and written informed consent was obtained by all participants prior to participation.
Chapter Six: Study 4 – Regional Sensitivities to Fatty Acid on the Human Tongue

6.3.3 Anthropometry

Body weight, height and BMI were measured as described in Chapter 2.6.

6.3.4 Fatty Acid Exposure to Taste Papillae Regions of the Tongue

A papillae stimulus test was conducted to identify which regions of the tongue are most susceptible to stimulation by C18:1 using a cotton bud. Two stimuli were included in the papillae stimulus test: C18:1 and paraffin oil. Paraffin oil was used as a control due to its absence of FFA, while mimicking the textural properties of C18:1. Participants were asked to protrude their tongue as a researcher administered stimuli on various locations of the tongue using a cotton bud (Figure 1.1). A stimulus of pure C18:1 or paraffin oil was applied to each papillae region of their tongue (fungiform, foliate and circumvallate), one at a time. Stimulus duration was 2s. After each stimulus application but before tongue was retracted, participants were asked whether they detected any taste sensation. A tongue depressor was used by the researcher to aid in applying stimuli to the circumvallate region of the tongue to prevent a gag-reflex. Stimuli were applied to each side of the tongue (left and right) separately. Applications of the stimuli (stimulus type, papillae region and tongue side) were conducted in a randomized order. Participants rinsed their mouth with water between each stimulation. A plain low-fat water cracker was provided halfway through testing (after 6 applications) and participants were given a 2-minute break to prevent sensory fatigue.
6.3.5 Fat Taste Threshold – Oral Cavity

FTT was measured using established methods (Haryono et al., 2014), as described in Chapter 2.4.1. For the purposes of this study, FTT were labelled as FFT-OC. An ascending series 3-AFC methodology was used, where participants were provided with multiple sample sets each containing three randomly-ordered samples of fat-free milk per set, two controls and one containing C18:1 at varying concentrations (0.02, 0.06, 1.00, 1.40, 2.00, 2.80, 3.80, 5.00, 6.40, 8.00, 9.80, 12.00, and 20.00mM). Participants were asked to taste each sample in the set and identify the sample that matched the taste quality from the warm-up sets, described further in Chapter 2.4.1. Correct identification of the C18:1 sample resulted in the participants repeating the same sample set. Incorrect identification of the C18:1 sample resulted in new sample set with a higher concentration of C18:1. FTT-OC was defined as the concentration of C18:1 correctly identified in three consecutive sample sets of the same concentration. FTT was transformed to an ordinal variable — FTT rank — ranging from 0 to 12, with higher ranks implying lower sensitivity to fat taste (Table 2.1).

6.3.6 Fat Taste Threshold – Fungiform Papillae

FTT of the fungiform papillae locus (FTT-F) was measured using a solid vehicle to validate the results found in the papillae stimulus test, as described in Chapter 2.4.2. Only the fungiform locus was measured in this test as foliate and circumvallate testing would cause discomfort in participants. Thresholds were measured using a triangle test with filter paper strips as a vehicle. Food grade C18:1 was added at varying volumes (0.25 to 50μL) to the tasting end of the filter paper strips. Paraffin oil was also added to the tasting end of the filter paper strips so that the total amount of
substrate equalled 50µL (e.g., the lowest concentration strip contained 0.25µL C18:1 and 49.75µL paraffin oil). Control strips contained 50µL paraffin oil.

Participants placed the tasting end of the filter paper strips on the tip of the tongue to taste the sample. A diagram was provided to participants to aid in placement of the strip. Samples were presented to the participants and completed in duplicate in the same manner as in the FTT method described in **Chapter 2.4.1**. The mean of the two final C18:1 volumes was recorded as FTT-F.

### 6.3.7 Statistical Analysis

Statistical analyses were conducted using computer software SPSS. Null hypotheses were rejected at $P < 0.05$. Descriptive statistics are reported as mean and SD, and categorical data presented as $n$ and %. The proportion of positive C18:1 taste responses (true positive) compared to positive paraffin oil taste responses (false positive) using a mixed model including taste response (positive or negative) as the dependent variable, and papillae region (fungiform, foliate or circumvallate), side of the tongue (left or right), substrate type (C18:1 or paraffin oil) and all double and triple interactions as fixed factors. The effect of fat taste sensitivity on responding positively or negatively to C18:1 was modelled using a mixed model including FTT-OC as the dependent variable, and substrate type, papillae region, taste response and all double and triple interactions as fixed factors. Participant ID was included as a random effect to prevent clustering of repeated measurements within participants. Post-hoc Sidak tests and $P$-values are reported for all analyses. A Spearman’s correlation ($R$) was conducted to assess test-retest reliability of the FTT-OC and FTT-F duplicate.
Characteristics of the participants are detailed in Table 6.1. Twelve (60%) participants in the sample were female.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>24.7 (6.2)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.6 (9.7)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>61.2 (12.0)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.4 (2.7)</td>
</tr>
</tbody>
</table>

Table 6.1 – Characteristics of participants from Study 4.

6.4.1 Papillae Stimulus Test

The proportion of positive C18:1 taste responses (true positives) and positive paraffin oil taste responses (false positives) were compared for both the left and right side of each papillae region (Figure 6.1). There were no significant differences in true positives between the left and right side of the tongue for any of the taste regions. There were significant differences between true positives and false positives in the foliate region for both the left \( (P < 0.001) \) and right \( (P = 0.002) \) side of the tongue. No differences between true positives and false positives were observed in the left or right side of the fungiform or circumvallate regions. In addition, more true positives were found in the foliate region compared to the fungiform \( (P < 0.001) \) and circumvallate regions \( (P = 0.010) \). There was no difference between true positives between foliate and circumvallate regions, or false positives between any of the papillae regions.
Figure 6.1 – Proportion of positive taste responses after C18:1 and paraffin oil stimulation at each papillae region, split by side of the tongue. ** $P$-value < 0.01, *** $P$-value < 0.001

To assess if fat taste sensitivity was associated with ability to detect C18:1, mean FTT-OC was compared between positive and negative C18:1 taste responses for each papillae region. There was a significant difference in FTT-OC between positive and negative C18:1 taste responses in the foliate region, and a small difference in the circumvallate region (Table 6.2). There was no difference in FTT-OC between positive and negative C18:1 responses for the fungiform region.
Chapter Six: Study 4 – Regional Sensitivities to Fatty Acid on the Human Tongue

<table>
<thead>
<tr>
<th>Papillae Region</th>
<th>Response</th>
<th>n</th>
<th>FTT-OC (mM)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungiform</td>
<td>Positive</td>
<td>10</td>
<td>13.0 ± 1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>22</td>
<td>12.8 ± 2.2</td>
<td>0.943</td>
</tr>
<tr>
<td>Foliate</td>
<td>Positive</td>
<td>24</td>
<td>6.9 ± 2.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>8</td>
<td>14.9 ± 1.4</td>
<td>0.007</td>
</tr>
<tr>
<td>Circumvallate</td>
<td>Positive</td>
<td>14</td>
<td>10.8 ± 1.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>18</td>
<td>15.6 ± 1.9</td>
<td>0.058</td>
</tr>
</tbody>
</table>

Table 6.2 – Comparison of FTT-OC in positive and negative C18:1 taste responders in each papillae region. Fat taste threshold in the oral cavity (FTT-OC) presented as mean ± standard error; n indicates the number of responses.

6.4.2 Fat Taste Threshold Tests

One participant was not able to differentiate the test sample at the highest concentration (pure C18:1) from the control samples in the FTT-F method, and therefore their FTT-F could not be determined and was excluded from this analysis.

The FTT-OC method was able to determine FTT-OC for all participants. The median FTT-OC was 14.9 (13.5 (interquartile range)) mM and FTT-F was 33.5 (45.0) µL.

There was strong test-retest reliability between duplicate measures of FTT-OC ($R = 0.877$, $P < 0.001$) and FTT-F ($R = 0.840$, $P < 0.001$). However, there was no correlation between mean FTT-OC and mean FTT-F ($R = -0.097$, $P = 0.731$).

6.5 Discussion

The aim of this study was to identify the regions of the tongue most susceptible to stimulation of fatty acid using a cotton bud. The foliate region was the most reliable region for detecting fatty acids with 75% of C18:1 applications being identified as having a taste response. This was significantly higher than fungiform (31%) and circumvallate (41%) regions. Folic TBCs express CD36 receptors in humans.
Chapter Six: Study 4 – Regional Sensitivities to Fatty Acid on the Human Tongue
(Simons et al., 2011), so it is reasonable that this region would be susceptible to fatty acid stimulation. In addition, participants who were able to detect C18:1 in the foliate region had a lower mean FTT-OC (6.9mM) compared to participants who were not able to detect C18:1 (14.9mM).

Human circumvallate TBCs express CD36 receptors (Simons et al., 2011; Zhang et al., 2011; Gilbertson et al., 2005), so it was expected that this region would respond positively to fatty acid stimulation. However, participants in this study were not able to differentiate C18:1 from the control in the circumvallate region. There was a difference in FTT-OC between positive taste responders (10.8mM) and negative taste responders (15.6mM), although statistical significance was not achieved ($P = 0.058$).

Both circumvallate and foliate TBCs express CD36 (Simons et al., 2011), but only circumvallate TBCs express FFAR4 (Galindo et al., 2011). There is limited evidence to suggest that there is coordination between CD36 and FFAR4 although in this study if FFAR4 and CD36 were required for a positive response to C18:1, we would expect circumvallate to be more responsive to the fatty acid than foliate (Ozdener et al., 2014). Also, C18:1 may have low specificity to receptors within circumvallate TBCs compared to other fatty acids, although this has not been investigated.

The fungiform region was not able to reliably differentiate C18:1 from the control in the papillae stimulus test. Also, FTT-OC was similar between positive (13.0mM) and negative (12.8mM) responders of C18:1 stimulation. These results were further validated by the results from the two different FTT tests. FTT-OC are known to correlate well with fat taste receptor expression levels (Galindo et al., 2012). However, FTT-F did not correlate with FTT-OC. This is due to the filter paper only targeting the fungiform locus whereas liquid media can be distributed throughout the oral cavity. While the relative expression of CD36 in human papillae is not known, rodent models
have shown lower expression of CD36 in fungiform TBCs (Gilbertson et al., 2005). Based on this, it is reasonable that the fungiform papillae region was less reliable for fatty acid detection. Expression of fat taste receptors within fungiform TBCs may vary among individuals which explains the large variation in FTT-F, but are not plentiful enough for reliable testing of human taste function.

It should be noted that this study only assessed fatty acid stimulation on papillae clusters using C18:1. A low response to C18:1 does not necessarily mean that will be the case for all fatty acids, although it has been reported that sensitivity to C18:1 taste correlates well with other fatty acids (Stewart et al., 2010; Newman & Keast, 2013). Also, this study only included a small number of participants which may not accurately represent variation within the total population.

6.6 Conclusion

The results from this study indicate that the foliate papillae region is the most sensitive region of the tongue for the detection of C18:1. It is recommended that when developing methods or treatments to assess fat taste sensitivity that a liquid medium is the best vehicle to use. Further, in methods using liquid vehicles, participants should ensure that samples cover the entire tongue including the back and sides, as the tip of the tongue may not be sensitive enough for a taste response. If a solid vehicle is necessary then the foliate papillae should be targeted rather than fungiform or circumvallate papillae.
Chapter Seven: Summary of Major Findings and Conclusions

7.1 Introduction

The worldwide prevalence of overweight and obesity is increasing rapidly (Aune et al., 2016; Ng et al., 2014), and has become a serious public health issue due to the association between obesity and increased risk of many chronic illnesses including type 2 diabetes mellitus (Abdullah et al., 2010), cardiovascular disease (Whitlock et al., 2009), and some cancers (Renehan et al., 2008). Although obesity is multifactorial and complex, one of the key contributors to the development of obesity is excess energy intake, particularly from high-fat, energy-dense foods (Hooper et al., 2015). Attenuated sensitivity to fat taste is a driver of excess fat intake due to reduced satiety response following fatty food consumption (Keast et al., 2014; Stewart et al., 2011b). The reason for the evolution of fat taste is likely twofold. First, it acts as a warning system to deter intake of fatty foods that have spoiled and are therefore high in FFA due to microbial hydrolysis of TG (Keast & Costanzo, 2015). Second, it aids in the regulation of energy intake due to satiety responses following fatty acid detection. However, fat taste sensitivity can be modulated by altering habitual dietary fat intake, with low-fat intake increasing sensitivity and high-fat intake decreasing sensitivity (Newman et al., 2016; Stewart & Keast, 2012). There are various negative health implications linked with impaired fat taste sensitivity. Individuals with impaired fat taste sensitivity are more likely to have a greater BMI, although this point is contentious with various human studies reporting this association (Asano et al., 2016;
Kindleysides et al., 2017; Stewart et al., 2010; 2011a; Martínez-Ruiz et al., 2014; Tucker et al., 2014); while others failing to find such association (Costanzo et al., 2017; Stewart & Keast, 2012; Running et al., 2013). A recent meta-analysis found no association between fat taste sensitivity and BMI, although the author could not conclude on these findings as it was only based on seven cross-sectional studies (Tucker et al., 2017). However, there is substantial evidence that that impaired fat taste sensitivity is associated with increase dietary fat consumption (Keast & Costanzo, 2015; Mattes, 2011), with some evidence to suggest that these associations are specific to saturated and monounsaturated fat sources (Costanzo et al., 2017; Running et al., 2015). This is concerning as high intakes of saturated fat lead to elevated levels of blood TG and cholesterol, which in turn is associated with increased risk of cardiovascular disease. The World Health Organisation (WHO, 2007), along with many national health authorities (DHHS, 2015; HC, 2011; NHMRC, 2006; PHE, 2016), recommend decreasing consumption of saturated fat, as this has been shown to reduce occurrences of cardiovascular events. As heightened sensitivity to fat taste is associated with reduced intake of total dietary fat and saturated fat (Keast & Costanzo, 2015; Mattes, 2011; Costanzo et al., 2017; Running et al., 2015), increasing sensitivity in taste impaired individuals may aid in the adherence to healthy low-fat diets. Fat taste sensitivity is not static, and is able to be modified via dietary fat intake (Newman et al., 2016; Stewart & Keast, 2012). The likely mechanism for this is downregulation of fat taste receptors following oral fat exposure and upregulation when oral fat is not present, although this has only been demonstrated in animal models. Both CD36 and FFAR4 are downregulated following dietary fat exposure in rodents (Gaillard et al., 2008; Laugerette et al., 2005; Martin et al., 2011; Zhang et al., 2011), with short-term fatty acid exposure having a greater effect on CD36 than FFAR4 (Martin et al., 2011).
Conversely, absence of dietary fat results in rapid upregulation of CD36 in rodents, back to pre-prandial levels following at least 11 hours of fasting, and gradual upregulation of FFAR4 (Martin et al., 2011).

There are many different SNPs for genes that are responsible for fat taste receptors (Liu et al., 2016). While these SNPs encode the same genes, their slight structural differences have an influence on an observable phenotype. For example, the A allele of SNP rs1761667 for CD36 has been associated with reduced fat taste sensitivity, increased creaminess perception, and liking of added fats in foods compared to the G allele in multiple populations (Keller et al., 2012; Melis et al., 2015; Mrizak et al., 2015; Pepino et al., 2012; Sayed et al., 2015). Also, the T allele of SNP rs1527483 for CD36 has been associated with increased perception of fat content compared to the C allele in African-American and Malaysian populations (Keller et al., 2012; Ong et al., 2017), but not in a Caucasian population (Melis et al., 2015). Beyond sensory perception, various SNPs for CD36 have also been associated with obesity, type 2 diabetes and reduced fat oxidation rate across multiple populations (Liu et al., 2016), showing a relationship between fat taste function and metabolic health. Similarly, various SNPs for the FFAR4 gene have been associated with increased obesity and reduced LCFA signal transduction (Ichimura et al., 2012; Waguri et al., 2013). While morphology of single genes have been shown to be associated with fat taste sensitivity, there is little information on the effect of genetics on fat taste sensitivity. One study that used a familial pedigree analysis reported that taste intensity ratings of C18:2 was 19% heritable (Garneau et al., 2017). However, intensity ratings are not an appropriate indicated of habitual fat intake as food rarely contains FFA in the supra-threshold range (Che Man et al., 1999; Koriyama et al., 2002). For reference, a twin study reported the heritability of the variability for detection thresholds to be 22% for salty.
taste and 53% for sour taste (Wise et al., 2007), providing potential that variation in detection threshold for other tastes may also be, at least in part, heritable.

The current gold standard for assessing fat taste sensitivity is by measuring fat taste threshold (Haryono et al., 2014; Heinze et al., 2015). Fat taste threshold is defined as the smallest amount of fatty acid exposed to the oral cavity necessary to elicit a detectable taste response, albeit with no taste quality attached (Keast & Roper, 2007). As the concentration of fatty acid increases, fat taste quality may then be tasted or recognised. Once the concentration of fatty acid is high enough for recognition and supra-threshold, the flavour is generally unpleasant. At the supra-threshold level, it is likely that sensory systems other than taste are involved, for example smell or chemesthesis, therefore fat taste quality is not equivalent to easily identified qualities such as sweet or salty (Keast & Costanzo, 2015). However, concentration of fatty acid in food at this level is uncommon, and is more likely to occur after spoiling of food. Therefore, detection threshold is the most useful taste dimension as a proxy for dietary fat intake. However, current methods to measure detection threshold, such as the triangle test (Stewart et al., 2011), 3-alternative forced choice (3-AFC) method (Haryono et al., 2014), 2-AFC method (Mattes, 2009) and the staircase method (Tucker et al., 2014), are lengthy to prepare and conduct particularly outside of a laboratory setting, require repeated measures to reduce false positives, and can cause sensory fatigue at high concentrations. In addition, the vehicles used in these methods, fatty acid in dairy (Haryono et al., 2014) or water (Tucker & Mattes, 2013) emulsions, are not stable for more than a few hours. Alternative vehicles have been proposed recently, such as fatty acid impregnated paper disks (Melis et al., 2015) and dissolvable taste strips (Garneau et al., 2017), although these have not been validated against the gold standard method (Haryono et al., 2014). Furthermore, these methods
Chapter Seven – Summary of Major Findings and Conclusions

rely on solid vehicles that may not stimulate all regions of the tongue as liquid vehicles do. Therefore is a need to develop and assess rapid measures of FTT.

7.2 Discussion of Major Findings

The aim of this thesis was to assess the influence of diet and genetics on fat taste sensitivity, which was carried out by recruiting adult MZ and DZ twin pairs into an 8-week dietary intervention trial. This thesis assessed the baseline associations between FTT and habitual fat consumption in the sample and also assessed baseline genetic contributions to fat taste using the classic twin model. Further, the thesis assessed the effect of 8-week dietary fat intake on modulation of FTT and fat taste receptor gene expression, and explored effect modification of zygosity on changes to FTT as a proxy for genetic effects. A secondary aim was to assess regional sensitivity of C18:1 on the tongue to aid in development of novel methods for measuring fat taste sensitivity. Discussions of the key findings from this thesis are as follows:

7.2.1 Fat Taste and Dietary Intake

This thesis aimed to assess the relationship between diet and fat taste sensitivity. It was hypothesised that increased fat intake would be positively associated FTT, and vice versa for decreased fat intake. Study 1 observed positive associations between FTT, dietary fat intake, and fatty food consumption; and Study 2 demonstrated modulation of FTT following controlled intake of dietary fat. These results indicate that fat taste sensitivity is regulated by a positive feedback system, where high fat consumption leads to further increases in fat intake. The ability to regulate fat taste sensitivity is likely an evolutionary adaptation, for example sensitivity may decrease...
when foods containing fat are abundant allowing overconsumption of the nutrient. In most positive feedback systems, the loop is broken once the stimulus is removed or suppressed (Crespi, 2004), for example if foods containing fat become less abundant. However, this becomes an issue in obesogenic environments where discretionary fatty foods are cheap and plentiful, which turns a positive feedback system into a vicious circle (Crespi, 2004). This may be causing a gradual shift towards attenuated sensitivity in developed populations where fatty food is abundant. Many studies that have measured fat taste sensitivity reported skewed distributions towards those who are less sensitive (Asano et al., 2016; Costanzo et al., 2017; Garneau et al., 2017; Kindleysides et al., 2017; Mattes, 2009; Newman et al., 2016; Pepino et al., 2012; Stewart et al., 2011a), although it is acknowledged that these distributions may also be a consequence of the concentration ranges used in the 3-AFC method. Regardless, if there is a gradual shift in attenuated sensitivity, this would likely have implications on fat intake in the population, particularly from saturated fat which may be a contributor to risk of cardiovascular disease (WHO, 2007). The current Australian and international dietary recommendations are to reduce saturated fat intake and to increase polyunsaturated fat in order to reduce risk of cardiovascular disease (DHHS, 2015; HC, 2011; NHMRC, 2006; PHE, 2016; WHO, 2007). While these recommendations are rational, they may not be practical in a population of individuals with impaired fat taste sensitivity. These individuals are more likely to consume greater amounts of saturated fat than polyunsaturated fat, as saturated fat had the greatest associations with FTT, ΔFTT and ΔFFAR4 expression compared with other fats, in Study 1, Study 2 and Study 3 respectively. Therefore, availability of discretionary fatty foods in the food supply should be considered when making health recommendations to a given population. Additionally, industrial reformulation of
foods to reduce the overall fat content and saturated fat content may be a solution to reduce accessibility of discretionary fatty foods which may aid in improving sensitivity to fat taste in the population, although food reformulation is met with its own set of challenges (Buttriss, 2013). Other sensory modalities add further complexity to this system. For example, the addition of saltiness to fatty food reduces the satiating effect of dietary fat, driving passive overconsumption regardless of fat taste sensitivity (Bolhuis et al., 2015), with growing evidence for the same to be true for the addition of sweetness to fatty foods (Bolhuis et al., 2018; Oliva et al., 2017). Also, decreased textural perception of fat is associated with increased consumption of discretionary fatty foods (Heinze et al., 2018). Therefore, public health initiatives aiming to reduce fatty food consumption should consider a multi-sensory approach to determine why individuals consume large quantities of fatty food before making dietary recommendations or food reformulation guidelines.

7.2.2 Fat Taste and Liking of Fatty Food

This thesis aimed to assess the influence of fat taste sensitivity on liking of fatty food. It was hypothesised that there would be no relationship between liking of fatty food and FTT as fatty acid is not normally found in foods in high enough quantity for recognition. Study 1 showed that FTT was not associated with liking ratings of fatty foods, liking ratings of reduced fat foods and the difference between these two ratings; and in Study 2, diet-mediated changes in FTT did not modify any of the food liking ratings. This is in line with a recent study that did not observe an association between fat taste sensitivity and liking of fatty food (Bolhuis et al., 2018). Rather, it is the other taste modalities in fatty foods, such as sweetness and saltiness, which have a larger effect on liking and consumption than TG content (Bolhuis et al., 2018; Oliva et al.,
This is likely because sweetness and saltiness are normally perceived at the supra-threshold, whereas detection and recognition thresholds are not associated with hedonics (Druz & Baldwin, 1982). Despite the findings from this thesis, some studies have reported that individuals with impaired fat taste sensitivity have greater preference for fatty foods (Asano et al., 2016; Bolhuis et al., 2015; Ledikwe et al., 2007; Mattes, 1993), although preference is not a great indicator of hedonics in this context as it does not evaluate the magnitude of difference in liking between food items. Nonetheless, it is interesting to see disparate results between similar studies. One explanation for this may be due variation in sensitivity to other flavour properties of fat: namely olfaction and texture. First, olfactory sensation of TG or fatty acid is not related to the gustatory sensation (Chalé-Rush et al., 2007; Kindleysides et al., 2017). As opposed to fat taste, fat olfaction does contribute to liking of fatty foods, for example anosmic mice have shown no preference for high-fat food over low-fat food whereas control mice have a higher preference for high-fat food (Kinney & Antill, 1996). However, a 24-week high-fat dietary protocol reduced olfactory sensation in rodents (Thiebaud et al., 2014), which is similar to the results of the 8-week dietary intervention on fat taste sensitivity in Study 2. It is possible that dietary fat interventions greater than 8 weeks may be able to modulate olfactory sensation, and therefore the liking of fatty foods. However these are conflicting dynamics, as a low-fat diet will increase fat taste sensitivity (reducing fat intake) but also increase fat olfaction sensitivity (increasing liking and preference for fat). Long-term dietary intervention studies are needed to assess the opposing nature of these two mechanisms in humans, and what the effect this has on overall fat intake. Second, textural sensation of TG is also not associated with taste sensitivity to C18:1 (Heinze et al., 2017). However, there is some evidence of oral texture sensitivity influencing fatty food
consumption. Individuals less sensitive to paraffin oil detection were more likely to consume servings of processed meats (Heinze et al., 2018). Despite this, oral texture sensitivity does not appear to be modifiable, as there was no change in TG perception following dietary intervention in Study 2. As with olfactory sensitivity, it is possible that longer dietary interventions are necessary to confirm this. Altogether, the results from this thesis suggest that the fat taste system does not contribute to the liking of fatty foods, but instead is mainly the result of olfactory properties of TG. Instead, impaired fat taste sensitivity drives the consumption of dietary fat via reduced satiety response to fatty food, both from impaired taste signalling (Keast et al., 2014) and reduced GIT response (Stewart et al., 2011b) independent of hedonics.

7.2.3 Fat Taste, Obesity and Satiety
This thesis aimed to assess the association between of fat taste sensitivity and anthropometric measures of obesity. It was hypothesised that there would be no relationship between FTT and obesity. In Study 1, there were no significant associations between FTT rank, BMI, waist circumference and waist-hip ratio. This is in line with previous studies (Bolhuis et al., 2015; Running et al., 2013; Stewart & Keast, 2012; Tucker et al., 2015) and a meta-analysis (Tucker et al., 2017), although some studies have reported a positive association between FTT and BMI (Asano et al., 2016; Martínez-Ruiz et al., 2014; Stewart et al., 2010; 2011a; 2011b). In addition, diet had a similar effect on FTT in participants who were and were not able to maintain body weight in over 8 weeks in Study 2, indicating that changes in body weight does not affect FTT. The results from this thesis suggest that obesity does not precede fat taste sensitivity. However, this does not mean that obesity does not result from fat taste sensitivity, although there is limited research in this area. Obesity is complex and
multifaceted, so there are many reasons why the effect fat taste sensitivity has on increased energy intake, and therefore obesity, remains contentious. First, while increased FTT does drive increased fat intake, there may be a compensatory effect with other macronutrients in some individuals in order to maintain total energy intake. This was observed in Study 1 by way of participants with high FTT rank consuming less energy from carbohydrate. Second, as there are many factors that influence energy intake and obesity, fat taste sensitivity is likely to contribute to only a small amount of total energy intake. There may indeed be a true influence of fat taste sensitivity on energy intake, but it may be too small to be detected in many of the above studies that were not able to find an association. However, small increases in energy intake are still important to note as they can have a large impact on weight status in individuals over long periods. With both these points in mind, the duration of the dietary assessment and anthropometry becomes increasingly important. There is some evidence for a causal effect of FTT on body weight presented in this thesis. In Study 2, participants were trained and encouraged to maintain weight throughout the 8-week trial with additional weight maintenance resources provided. Despite these efforts to maintain body weight, 11 individuals lost significant weight on the low-fat diet. This demonstrates the difficulty of maintaining weight under a low-fat dietary protocol in free-living individuals, and weight loss was likely driven by the gradually decreasing FTT in these participants. This is also supported by previous intervention studies that did not aim to maintain weight, which reported individuals on low-fat dietary protocols lost significant amount of weight (Newman et al., 2016; Stewart & Keast; 2012). At the time of publication, the authors of these studies (Newman et al., 2016; Stewart & Keast; 2012) could not disassociate whether decreasing FTT was causal to weight loss or vice versa. In conjunction with the results from Study 2 which indicate
that weight loss does not affect FTT, these three intervention studies provide strong evidence that reduction in FTT does lead to successful weight loss. However, no long-term follow-up studies have been conducted on individuals who participated in FTT reduction. Therefore, there is a need for long-term studies to assess the effect of fat taste sensitivity on weight loss retention over long periods.

There is reasonable evidence that satiety is the mechanism that causes fat taste sensitivity to drive energy intake. A satiety cascade occurs when the oral cavity is exposed to dietary fat (Keast & Costanzo, 2015) where the body readies itself for fat digestion with increased plasma TG mobilisation (Mattes, 2001a; 2001b); lipase secretion (Wojdemann et al., 1997); transient stimulation of GIT hormones, including cholecystokinin, pancreatic polypeptide and peptide YY (Robertson et al., 2001; Wisén et al., 1992); variations in postprandial glucose and insulin (Robertson et al., 2001; Chavez-Jauregui et al., 2010); and thus an increased feeling of fullness (Little & Feinle-Bisset, 2011; Smeets & Westerterp-Plantenga, 2006). It likely that the satiety response following oral dietary fat exposure is paralleled by satiety responses throughout the alimentary canal, as individuals with impaired fat taste also have reduced plasma cholecystokinin, peptide YY and appetite following duodenal C18:1 infusion (Stewart et al., 2011b). A study by Keast et al. (2014) showed that individuals classed as hyposensitive to fat taste found a high-fat meal to be less satiating and had a greater intake of dietary fat and energy in an acute eating situation compared with hypersensitive individuals. In this way, an individual who has impaired sensitivity due to adaptation to a high-fat diet will feel less full and consume greater quantities of energy. While this thesis did not conduct any formal measures of satiety, Study 3 demonstrates regulation of fat taste receptor gene expression following habitual dietary fat intake. Particularly, there was increased FFAR4 expression following low-
fat dietary intake and decreased expression following high-fat dietary intake. There is considerable evidence that activation of *FFAR4* by fatty acid stimulates a satiety response in both the oral cavity (Martin et al., 2011; 2012) and throughout the GIT (Hirasawa et al., 2005). The function of *FFAR4* is mediated via the release of a class of satiety hormones called incretins, such as glucagon-like peptide 1 and gastric inhibitory peptide (Tanagho & Shohdy, 2016). Therefore, increased expression of *FFAR4* in the oral cavity, and presumably throughout the alimentary canal, following low dietary fat intake leads to greater incretin secretions following fatty acid stimulation (Hirasawa et al., 2005), and potentially other satiety hormones as well. This may justify the reduced energy intake and weight loss observed in the participants on the low-fat diet in Study 2. The results also suggest that *CD36* is less important as a mediator of long-term satiety, as while the results from Study 3 showed expression of *CD36* trended in a similar direction to *FFAR4*, the changes in *CD36* expression were relatively small. Due to the rapid downregulation of CD36 following oral fatty acid exposure and subsequent return to ‘normal’ levels after fasting (Martin et al., 2011), *CD36* may be involved in mediating short-term satiety throughout an acute eating event, regardless of an individual’s habitual dietary fat intake. Therefore, *FFAR4* may be a better potential target for weight loss treatments and therapies. Low-fat dieting may aid in increasing expression of *FFAR4* throughout the alimentary canal leading to increased post-ingestive satiety response to fatty food and reducing passive overconsumption. Diets that approach the lower AMDR for fat (WHO, 2007) are likely to be useful in Western populations to aid in lowering energy intake and risk of obesity.
7.2.4 Fat Taste, Gene Expression and Genetics

This thesis aimed to assess the influence of diet on fat taste receptor gene expression. It was hypothesised that decreased fat intake would upregulate gene expressions of CD36, FFAR4, and KCNA2, and vice versa for increased fat intake. As demonstrated by Study 2 and other dietary intervention studies (Newman et al., 2016; Stewart & Keast 2012), habitual intake of dietary fat modulates fat taste sensitivity. Rodent studies have shown that changes in dietary fat intake regulates levels of CD36 and FFAR4 in the oral cavity (Martin et al., 2011; Zhang et al., 2011) which is likely the mechanism for changes in fat taste sensitivity, although this had never been assessed in humans. Study 3 showed a significant increase in FFAR4 expression in human fungiform papillae tissue following 8 weeks of low dietary fat intake. There were also significant associations between FFAR4 expression and intakes of total fat, saturated fat, monounsaturated fat and polyunsaturated fat, indicating that fatty acids with any level of saturation may downregulate FFAR4 expression. In addition, the change in FFAR4 expression was associated with change in FTT, or in other words as expression increased, fat taste sensitivity also increased. However, the time-diet interaction for FFAR4 expression was weak \((P = 0.063)\) despite a strong time-diet interaction for FTT \((P < 0.001)\). The reason for this is likely because of a lack of power due to the small amount of participants in this study. Also, it should be noted that the gene expression analysis was conducted using fungiform papillae tissue. FFAR4 is expressed in human circumvallate papillae (Galindo et al., 2011), and while the expression of FFAR4 in human foliate papillae is unknown, it has been shown to be expressed in mouse foliate papillae (Cartoni et al., 2010) so it is likely to be present in humans as well. Also, the relative expression of FFAR4 between gustatory papillae is not known. Study 4 showed that the fungiform papillae cluster was not a reliable
region for fatty acid detection compared with other papillae clusters, particularly foliate. Therefore, much of the changes in FFAR4 expression may have been occurring in circumvallate and foliate papillae TBCs which were largely responsible for the strong change in FTT. Therefore, assessment of the effect of dietary intake in foliate and circumvallate tissue is recommended, although access to these papillae tissue are extremely limited in living humans. Another reason for the disparity between time-diet interactions for FFAR4 and FTT may be due to other receptor genes involved in fat taste function being responsible for the shift in FTT. However, none of the other gene expressions that were assessed had evidence for a significant time-diet interaction. CD36 is often recognised as the principal fat taste receptor although it seems to function and be regulated independently to FFAR4 (Martin et al., 2011). However, there may be coordination and interaction of between FFAR4 and CD36 via intracellular signal transduction (Abdoul-Azize et al., 2014), and upregulation of both these receptors in tandem may have a greater effect on fat taste sensitivity than either one individually. In Study 3, CD36 expression trended in the hypothesised direction similar to FFAR4, in that expression increased in the low-fat diet and decreased in the high-fat diet after 8 weeks. However, these changes in CD36 expression were relatively small. Again, this may be due to the analysis being on gene expression in fungiform papillae tissue rather than foliate or circumvallate, as shown in Study 4 that fungiform papillae clusters are not as sensitive to fatty acid detection compared with circumvallate and foliate. In addition, a study in mice demonstrated that CD36 expression was lower in fungiform TBCs compared with other gustatory papillae TBCs (Gilbertson et al., 2005). However, it should also be noted CD36 rapidly returns to pre-prandial levels following at least 11 hours of fasting (Martin et al., 2011). As the fungiform papillae biopsy was conducted in participants who had fasted
overnight, it is possible that expression of CD36 had returned to ‘normal’, which was why a significant time-diet interaction was not observed. As discussed in Chapter 7.2.3, it is speculated that the role of CD36 is to mediate short-term satiety during an acute eating event, while FFAR4 is involved in regulating long-term satiety after consuming dietary fat. Analysis of CD36 expression in human papillae immediately before and after consumption of dietary fat is necessary to confirm this. Similar to CD36 expression, there was a small increase in KCNA2 expression after 8 weeks of low dietary fat intake. This may have been due to changes in polyunsaturated fat intake, as there was evidence for an association between Δ KCNA2 and Δ polyunsaturated fat intake, which is reasonable as KNCA2 encodes the receptor responsible for PUFA detection (Liu et al., 2016). While the fatty acid used in the FTT measure in Study 3, C18:1, is not able activate KCNA2 as it is a monounsaturated fatty acid, there was evidence for a weak negative association between KCNA2 expression and FTT, similar to FFAR4. This may be because detection thresholds between C18:1 and C18:2 are highly correlated (Stewart et al., 2010). It seems that KCNA2 expression and PUFA taste function are regulated by polyunsaturated fat intake, independent of saturated and monounsaturated fat intake. The SCFA receptor genes FFAR2 and GPR84 were not regulated by changes in dietary fat intake, which is reasonable as they dietary fat contains little SCFA. However, there was a significant association between Δ FFAR2 and Δ dietary fibre intake, and GPR84 and energy from dietary fibre. These are interesting associations, as increased dietary fibre intake leads to increased production of SCFA by biota in the GIT (Kles & Chang, 2006). Since regulation of taste receptors is presumably analogous throughout the alimentary canal (Stewart et al., 2011b), it is possible that increased exposure to SCFA in the GIT may cause of the increased expression of lingual FFAR2 and GPR84. Overall, the results
from this thesis indicate that fasting $\text{FFAR4}$ and $\text{KCNA2}$ expressions are regulated by habitual total fat and polyunsaturated fat intake, respectively, but long-term dietary fat intake does not affect fasting $\text{CD36}$ expression.

This thesis also aimed to assess the heritability of FTT, and explore the effect modification of zygosity on diet-mediated change to FTT as a proxy for genetic effects. It was hypothesised that a proportion of the variation in FTT would be heritable based on evidence that detection thresholds for other taste modalities had some degree of heritability. There is limited research on the heritability of detection thresholds. A twin study by Wise et al., (2007) reported a heritability estimate of 22% for salty detection thresholds, which is a low level of heritability indicating that there is high inter-variability. Heritability estimate of sour detection thresholds were moderate at 53%, which indicates a lower inter-individual variability compared with salty detection thresholds. The heritability estimate of FTT, as reported in Study 2, was 8%, which is very low compared to other tastes. However, this low level of heritability is reasonable as many studies, including Study 1, have reported large inter-individual variation in FTT (Asano et al., 2016; Bolhuis et al., 2015; Costanzo et al., 2017; Martínez-Ruiz et al., 2014; Running et al., 2013; Stewart et al., 2010; 2011a; 2011b; Tucker et al., 2015), some as large as 20-fold differences in concentration. In addition to the low heritability estimate of FTT, ICC of MZ and DZ twin pairs were similar. This suggests that there is some factor that is causing similarity within twin pairs, but this factor does not have a genetic component. It is likely that the factor is familial environment, such as dietary intake, which supports the results from Study 2 and Study 3, where dietary fat intake had a strong effect on the regulation of FTT and fat taste receptors. To this point, there was no effect modification of zygosity on changes to FTT. In other words, the pattern of change in FTT following the diets was
similar in within MZ pairs as it was within DZ pairs, suggesting that genetic effects do not have an influence on an individual’s ability to modify FTT. This is an important detail, as it means that strategies or therapies targeting a reduction in fat taste sensitivity to increase satiety and reduce energy intake will be equally as effective to an entire population, at least from a genetic perspective. This adds supplementary weight to the argument for recommending low-fat dietary protocols in order to increase fat taste sensitivity, and therefore aid in lowering energy intake and risk of obesity.

7.4 Future Directions
This thesis has highlighted the influence of diet and genes on fat taste sensitivity, and the role fat taste may have on risk of obesity. However, the clinical applications of fat taste function in reducing fat intake are not widespread or evident. Results from this thesis may provide some understanding on how the mechanisms of fat taste may be manipulated to increase satiety and reduce fat intake, and subsequently energy intake and risk of obesity. First, there is a need for more efficient methodology to assess fat taste sensitivity. Nearly all published studies on fat taste sensitivity have used the gold standard method (Haryono et al., 2014), or variations thereof, to measure FTT (Asano et al., 2016; Bolhuis et al., 2015; Costanzo et al., 2017; Kindleysides et al., 2017; Martínez-Ruiz et al., 2014; Running et al., 2013; Stewart et al., 2010; 2011a; 2011b; Tucker et al., 2015). However, these methods, while accurate (Newman & Keast, 2013), have a significant time burden on participants and researchers, up to an hour to assess an individual FTT when completed in duplicate. When tasting many repeated samples, especially at higher concentrations, there is a carryover effect of the tastant.
Also, the fatty acid-in-dairy or -water emulsion used in this method is relatively unstable lasting for only a few hours, which makes this method difficult to conduct outside of a controlled laboratory setting. The idea of fatty acid impregnated paper disks (Melis et al., 2015) and dissolvable taste strips (Garneau et al., 2017) were promising as they are relatively stable and may have a reduced impact on sensory-specific satiety. However, the results from Study 4 have shown that there is high variability of fat taste perception between papillae clusters on the tongue. Therefore, solid vehicles may not be appropriate as the location that are placed on the tongue may have an effect on fatty acid detection. Liquid vehicles are most suitable in this case, although the use of more stable emulsifiers needs to be explored. Further to this point, terminology of fat taste and needs to be unified to aid in the understanding and interpretation of this taste sensation. There is currently no agreed upon term for fat taste, with alternative terms including fatty acid taste (Kindleysides et al., 2017), oleogustus (Running et al., 2015), and pinguis (Reed & Xia, 2015), which adds to the complexity of understanding the unique taste. While the term fat taste may be misinforming, especially to non-scientific audiences, as it does not refer to taste perception to all of dietary fat but rather to just fatty acids, one could argue that fat taste does not exist outside of the consumption of dietary fat and has a direct role in the regulation of fat intake. Regardless, a uniform term for fat taste is necessary for the wider acceptance and understanding of fat as an independent taste sensation.

The relationship between fat taste sensitivity and fat intake is clear, as evidenced by Study 1 and Study 2. However, the link between fat taste sensitivity and energy intake, and subsequently obesity, is not clear. A meta-analysis of the literature reporting that there is no association between fat taste sensitivity and obesity, albeit only seven studies used in the analysis (Tucker et al., 2017). However, Study 2
showed a decrease in body weight following a low-fat diet protocol and increased fat
taste sensitivity, despite efforts to maintain energy intake and body weight. There is a
need to further understand this association, as if fat taste sensitivity does indeed drive
consumption of energy, even if minute, this may be of relevance to clinical treatment
of overweight and obesity. This is coupled with the need to further understand the link
between fat taste sensitivity and satiety, as reduced satiety response is likely the driver
for excess energy consumption in individuals with impaired fat taste sensitivity. While
it has been reported that fat taste sensitivity is associated with acute energy intake and
satiety (Keast et al., 2014), the implications of fat taste sensitivity on habitual and
long-term dietary intake are not known. Longitudinal prospective studies may be
necessary to confirm the link between fat taste sensitivity and obesity, although this
type of research takes a long time to conduct.

While there is a clear narrative for the influence of fat taste sensitivity on fat intake, it
becomes increasingly more complex when considering additional taste modalities.
Fatty foods are rarely consumed in isolation of other tastes, and these tastes have a
marked impact on appetite independently as well as in combination with fat. For
example, saltiness, and perhaps sweetness, override the satiety response to fatty food
consumption in individuals regardless of their fat taste sensitivity (Bolhuis et al., 2015;
2018). The physiological mechanism of these interactions are unclear and should be
the target of future research on appetite and dietary fat intake. Interactions with other
promotor tastes should also be considered, including umami and the recently
discovered complex carbohydrate taste (Low et al., 2017).

Fatty acid intake (Panickar & Bhathena, 2010) and lower taste thresholds (Sakai et al.,
2017) have been associated with reduced incidence of neurological disorders in elderly
populations, specifically semantic dementia and Alzheimer disease. There is potential
for fat taste function to have a role in preventing degenerative neurological disorders via regulating the intake of beneficial fatty acids. While there is currently very limited research in this area, there is potential for fat taste function to have a role in maintaining human health beyond obesity.

Understanding the regulation of fat taste receptor gene expressions is critical to the development of oral therapies and treatments to increase sensory-specific satiety to fat. As reported in Study 3, long-term dietary fat intake regulates FFAR4 but not CD36. This may be because CD36 displays a diurnal rhythm of expression, rapidly downregulating following oral exposure to fatty acid, and returning to ‘normal’ levels following at least 11 hours of fasting (Martin et al., 2011). This suggests that the role of CD36 is to regulate short-term satiety and fat intake. Knowing this, it may be possible to activate CD36 and other receptors using fatty acid exposure as a treatment prior to a meal, which may reduce pre-prandial hunger and subsequent energy intake during the meal, especially if the meal contains fat. It is recommended that the treatment be delivered by a liquid vehicle, such as a mouth wash, as Study 4 showed large variations in fat taste perception between papillae clusters. This would be an interesting study, because if pre-prandial activation of fat taste receptors does lead to reduced energy intake, then it may be an effective strategy to suppress appetite and decrease body weight. One thing that should be noted is that this may be effective in the short-term, but may lose efficacy if FFAR4 adapts and downregulates following long-term stimulation from fatty acid. Ideally, the reduction in acute fat intake from the activation of CD36 would offset the increase in oral fatty acid exposure from the treatment. Alternatively, if an agonist for CD36 but not FFAR4 could be developed, or if fatty acid could be administered in the presence of a FFAR4 antagonist, then the treatment would be effective triggering a satiety response prior to a meal and reducing
excessive consumption without impacting long-term expression of FFAR4. Another thing that should be noted is that there would be a dose response to the treatment, where less sensitive individuals would need a higher dosage. Future studies that assess each of these aspects would be beneficial to the advancement and understanding of fat taste, as fat taste receptors may be potential targets for obesity therapies and treatments.

7.5 Conclusions

Major conclusions from this thesis are as follows:

- Habitual intake of dietary fat is the main influencer of fat taste sensitivity, with minimal effect of genetic factors on fat taste sensitivity. Low dietary fat intake increases sensitivity and high dietary fat intake attenuates sensitivity. This is further supported by the low heritability estimates which indicate high variability of FTT within the population, likely due to large differences in fat intake between individuals.

- Changes in fat taste sensitivity following dietary intervention are the result of regulation of fat taste receptor gene expression, particularly FFAR4 which is likely involved in the mediated long-term dietary fat intake. A greater expression of FFAR4 would trigger a greater satiety response following activation by fatty acid, leading to reduced intake of fatty food. KCNA2 may also support in regulating intake of polyunsaturated fat. CD36 was not involved in regulating long-term dietary fat intake and more likely to function as a mediator of short-term satiety following acute dietary fat intake.
Manipulating gene expression and receptor activation may be novel solutions for moderating satiety and reducing risk of overweight and obesity.

- Baseline associations between fat taste sensitivity and dietary fat intake suggest that less sensitive individuals are more likely to consume greater amounts of fat, particularly from monounsaturated and saturated fat sources. This finding is consistent with the previous research that has identified this association. Contrary to this, there was no direct association found between fat taste sensitivity and obesity, suggesting that any effect on energy consumption is small or there are weight regulation mechanisms that compensate for changes in fat intake.

- Individuals with impaired fat taste sensitivity did not necessarily have a greater liking for fatty foods, or were they any less able to perceive TG content in a food matrix compared with sensitive individuals. Furthermore, changes in dietary fat intake did not affect liking or TG perception. These results suggest that increased fat intake is not driven by liking or TG perception, but rather due to the magnitude of satiety response following fatty food consumption, where individuals with impaired fat taste sensitivity have reduced satiety response and therefore passively overconsume fatty foods. It is important to note that the combination of other taste modalities, particularly salty, may override the satiety response generated from fatty food consumption.

- Perception of fatty acid was not equal between papillae clusters on the tongue, where foliate papillae clusters appeared to be the most sensitive region. Development of novel fat taste sensitivity measures should consider these regional sensitivities. Altogether, these data suggest the potential for future
liquid oral treatments to stimulate fat taste receptors in order to increase satiety and reduce passive overconsumption of fatty foods.
Chapter Eight: References


National Health and Medical Research Council (NHMRC), 2006. Nutrient reference values for Australia and New Zealand including recommended dietary intakes. Canberra, Australia: NHMRC.


Appendices

Appendix A – Screening Form (Study 1, Study 2 and Study 3)

Screening form

Subject ID________

Date:________

1. D.O.B:_______ Age______ (include 18-70 years)

2. Male or female:________________

3. Estimated weight (kg):________

4. Estimated height (metres):_______

5. Estimated BMI (kg/m$^2$):________ Healthy (BMI 18.5-25) Yes/No

                      Overweight (BMI >25) Yes/No

6. Do you currently smoke? Yes/No

7. If no to question 9, were you previously a smoker?

8. If yes to question 7, when did you stop smoking?________________________________________

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

If yes, provide details__________________________________________________________

10. Will you be able to make the dietary changes for 8 weeks? Yes/No

11. Will you be able to consume either the low fat diet or moderate fat diet? Yes/No

12. Will you be able to taste test a variety of different foods including milk, custard, mousse, cream cheese, peanut butter and salad dressings? Yes/No
13. Are you happy to provide a saliva sample?  Yes/No

14. Are you able to attend Deakin University at the Burwood Campus for a total of 3 appointments?  Yes/No

*Note attendance at Deakin University is required at the beginning, week 4 and week 8 of the dietary intervention

Subject eligible. Yes/No

If no, provide reason_________________________________________

CONTACT DETAILS

Name: __________________
Address: __________________________________________________
________________________________________________
________________________________________________
Home Phone:____________________
Work Phone:_____________________
Mobile:____________________
Email: __________________________
Best time/day for contact: ________________________________
Testing session booked or TBA?____________________
Appendix B – Twin Registry Australia Recruitment Letter (Study 1, Study 2 and Study 3)

Dear Twin Registry Member,

Thank you for your continued membership of the Australian Twin Registry. Enclosed is a letter inviting you and your twin to participate in important new research looking at **why some people over consume fatty foods**, being conducted by A/Prof Russell Keast from the School of Exercise and Nutrition Sciences at Deakin University.

The enclosed letter from the above investigator provides you with a general description of his study, *Why do some people overconsume fatty foods?*, and explains in detail the procedures involved in participating in the project, including the approximate amount of your time that would be required and any benefits or consequences that may be the result of your involvement.

This project has been independently reviewed and approved by the Australian Twin Registry as being of significant scientific value to the nominated area of study and meeting the necessary ethical requirements.

**Responding to this invitation**

The ATR appreciates receiving all responses to our study invitations. If possible please return the enclosed response form, whether you wish to participate or not, **within the next 2 weeks**. Alternatively please call us on 1800 037 021. Please note that you may receive a reminder via mail or telephone if we have not heard from you within this timeframe.

If you need changes made to your address and/or other details, please note these on the form or phone us on Freecall 1800 037 021 and we will update our records.

**Ethics and Confidentiality**

If you have a complaint regarding any aspect of the research project or the Australian Twin Registry you may contact any or all of; the Australian Twin Registry on 1800 037 021; the Executive Officer of the Human Research Ethics Committee at the University of Melbourne on 03...
8344 2073 or; the Executive Officer of the Human Research Ethics Committee at Deakin University on 03 5227 2368.

Participation in this or any Twin Registry study is voluntary. All information concerning prospective and confirmed participants remains strictly confidential. This means that access to information that identifies you will be made available to the researchers only if you and your twin have agreed to take part in the study. Any confidential data resulting from your participation in the study will be kept securely in the files of the Twin Registry and the researchers. It will not be passed on to anyone else without your permission. Research data from the results of the study may be published but will contain no identifying information relating to the individual participants. You are always free to refuse to participate in this research program, and can withdraw at any stage.

We appreciate your having taken the time to consider this request and thank you in anticipation of receiving your response.

Yours sincerely,

[Signature]

Professor John Hopper, Director
Participants needed!

We are looking for non-smoking volunteers 18-50 year, normal weight (BMI between 18.5 – 25 kg²/m) to participate in a study that looks at taste preferences.

**Participation involves:**

- Attending three sessions at the Burwood campus to taste foods with various fat, salt, and sugar contents (Sept/Oct 2015)

- Completing questionnaires on your dietary habits and for females questionnaire about menstrual cycle and contraception.

**All participants will receive a $50 Coles/Myer Voucher**

**Contact for more information:**

Email [sensory@deakin.edu.au](mailto:sensory@deakin.edu.au) or call (03)92468215
## Appendix D – Fat Taste Threshold Data Collection Form

### Fat Detection Threshold Test

<table>
<thead>
<tr>
<th>Concentration (mM)</th>
<th>Sample Number</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.06</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
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<tr>
<td>2.8</td>
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<tr>
<td>3.8</td>
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<tr>
<td>5</td>
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<tr>
<td>6.4</td>
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<tr>
<td>8</td>
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<td></td>
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</tr>
<tr>
<td>9.8</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
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</tr>
</tbody>
</table>

### Duo Trio Test (20mM)

Correct detection? _______
Appendix E – Triglyceride Ranking Score Data Collection Form

Participant ID number:  
Testing date:  
Testing week of diet:  

Fat Ranking Test  
Fat levels  
0% = 934  
2% = 609  
6% = 245  
10% = 957  

Ranking order  

_______   >   _______   >   _______   >   _______  

Score  

_______
Appendix F – Food Liking Data Collection Form*

Participant ID number:
Testing date:
Testing week of diet:

Please write the sample number in the space provided. For each set of samples, please taste and rate each sample individually and rate your liking on the scale.

Sample number ____

Sample number ____

*Form adapted for use on Compusense.
## Appendix G – Like-Dislike Questionnaire

### Participant ID number:

### Background

This questionnaire is designed to assess your like or dislike of a number of food/beverage items and common experiences.

### Confidentiality

All the information provided in this questionnaire will be treated in the strictest confidence.

### How to fill in the questionnaire

You will rate your like or dislike of a number of food/beverage items and common visual, smell and sound experiences. For each item, please clearly place a vertical line on the scale at the point that best represents your like or dislike of that item.

The centre of the scale is neutral (0); you neither dislike nor like the item. The left side of the scale represents dislike (-100), whilst the right side of the scale represents like (100).

Below is the scale you will use to rate your like or dislike of foods and common experiences. You will rate each item on a separate scale.

<table>
<thead>
<tr>
<th>Strongest Imaginable Dislike</th>
<th>Neutral</th>
<th>Strongest Imaginable Like</th>
</tr>
</thead>
<tbody>
<tr>
<td>-100</td>
<td>Strong</td>
<td>Like</td>
</tr>
<tr>
<td>Very strong</td>
<td>Moderate</td>
<td>Very strong</td>
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<tr>
<td>Strong</td>
<td>Weak</td>
<td>Strong</td>
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<tr>
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<td>Moderate</td>
<td>0</td>
</tr>
<tr>
<td>Very weak</td>
<td>Moderate</td>
<td>Very weak</td>
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<tr>
<td>Very strong</td>
<td>Weak</td>
<td>Very strong</td>
</tr>
</tbody>
</table>

The scale ends i.e. ‘strongest imaginable dislike’ and ‘strongest imaginable like’ represent the strongest liking or disliking of any kind. For example, the sensation of walking out of a dark movie theatre on a bright sunny day would be rated by most people somewhere between ‘moderate’ and ‘strong’ disliking. Although it may be an unpleasant experience, many people would not consider this as the strongest disliked experience. Please think about this when providing your answers.

Please also keep in mind you will be rating your like or dislike of not only foods and beverages, but also common experiences so, for example, you like the smell of a fresh rose more than chocolate, you would give it a higher rating on the scale.

If you have never eaten a particular food, or never experienced one of the listed items, please rate the item as ‘neutral’.

Please turn this page over to begin the questionnaire.
Section one: Foods and beverages

Listed below are a number of food/beverage items. Please rate how much you like or dislike the food/beverage by clearly placing a vertical line on the scale.

a) Asparagus

b) Blueberries

c) Broccoli

d) Cantaloupe

e) Carrot
f) Grapefruit juice

-100 Very strong
- Strong
Weak
Moderate

0 Neutral
Moderate

Strong
Very strong
100

Strongest Imaginable
Dislike
Like

g) Plain porridge

-100 Very strong
- Strong
Weak
Moderate

0 Neutral
Moderate

Strong
Very strong
100

Strongest Imaginable
Dislike
Like

h) Strawberries

-100 Very strong
- Strong
Weak
Moderate

0 Neutral
Moderate

Strong
Very strong
100

Strongest Imaginable
Dislike
Like

i) Sweet potato

-100 Very strong
- Strong
Weak
Moderate

0 Neutral
Moderate

Strong
Very strong
100

Strongest Imaginable
Dislike
Like

j) Tomato juice

-100 Very strong
- Strong
Weak
Moderate

0 Neutral
Moderate

Strong
Very strong
100

Strongest Imaginable
Dislike
Like

k) Wholemeal bread

-100 Very strong
- Strong
Weak
Moderate

0 Neutral
Moderate

Strong
Very strong
100

Strongest Imaginable
Dislike
Like
Appendices

l) Burn of a spicy meal

m) Beef steak

n) Butter

o) Cookies

p) Hot chips

q) Fried chicken
r) Buttercream icing

<table>
<thead>
<tr>
<th>Strongest Imaginable</th>
<th>Neutral</th>
<th>Strongest Imaginable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dislike</td>
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<td>Like</td>
</tr>
<tr>
<td>-100</td>
<td>Very strong</td>
<td>0</td>
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</tbody>
</table>

s) Mayonnaise

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<th>Strongest Imaginable</th>
<th>Neutral</th>
<th>Strongest Imaginable</th>
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<tbody>
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<td>Dislike</td>
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<td>Like</td>
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<tr>
<td>-100</td>
<td>Very strong</td>
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t) Sausage

<table>
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<tr>
<th>Strongest Imaginable</th>
<th>Neutral</th>
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<tbody>
<tr>
<td>Dislike</td>
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<td>Like</td>
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<td>-100</td>
<td>Very strong</td>
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</tbody>
</table>
Section 2: Common experiences

Listed below are a number of common visual smell and sound experiences. Please rate how much you like or dislike the experience by clearly placing a vertical line on the scale.

a) Buzz of lights

b) Smell of cut grass

c) Glare of headlights

d) Walking on hot pavement

e) Cold wind in your face
f) Jumping in a pool on a hot day

<table>
<thead>
<tr>
<th>Strongest Imaginable</th>
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g) Sound of a child laughing

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<tr>
<th>Strongest Imaginable</th>
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<tbody>
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h) Light sparkling on water

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</table>

i) New car smell

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<thead>
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<td>Weak</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Strong</td>
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j) Feel of a soft blanket

<table>
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<tr>
<th>Strongest Imaginable</th>
<th>Neutral</th>
<th>Strongest Imaginable</th>
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<tbody>
<tr>
<td>Dislike</td>
<td>Strong</td>
<td>Like</td>
</tr>
<tr>
<td>-100</td>
<td>Very strong</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Weak</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Strong</td>
<td></td>
</tr>
</tbody>
</table>

Please bring this completed questionnaire with you on the day you visit the Deakin University sensory laboratory.

Thank you for completing this questionnaire.
Appendices

Appendix H – Foods used in Hedonic Rating Test

### Savoury Biscuit

<table>
<thead>
<tr>
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<th>Nutrition Information</th>
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<tbody>
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<tr>
<td>Servings per package:</td>
<td>9</td>
<td>Servings per package:</td>
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<tr>
<td>Serving Size:</td>
<td>25g</td>
<td>Serving Size:</td>
<td>25g</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Quantity</strong></td>
<td><strong>Per Serving</strong></td>
<td><strong>Quantity</strong></td>
<td><strong>Per 100g</strong></td>
</tr>
<tr>
<td>Energy</td>
<td>493kJ</td>
<td>1970kJ</td>
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<tr>
<td>Protein</td>
<td>2.0g</td>
<td>7.9g</td>
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</tr>
<tr>
<td>Total Fat</td>
<td>5.0g</td>
<td>20.0g</td>
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</tr>
<tr>
<td>-Saturated</td>
<td>1.0g</td>
<td>3.9g</td>
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</tr>
<tr>
<td>Carbohydrate</td>
<td>15.6g</td>
<td>62.6g</td>
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<tr>
<td>Sodium</td>
<td>212mg</td>
<td>848mg</td>
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### Peanut Butter

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<td>Servings per package:</td>
<td>22.7</td>
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<td>Serving Size:</td>
<td>22g</td>
</tr>
<tr>
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<td></td>
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<td></td>
</tr>
<tr>
<td><strong>Quantity</strong></td>
<td><strong>Per Serving</strong></td>
<td><strong>Quantity</strong></td>
<td><strong>Per 100g</strong></td>
</tr>
<tr>
<td>Energy</td>
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<td>2590kJ</td>
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<tr>
<td>Protein</td>
<td>5.2g</td>
<td>23.8g</td>
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<td>Total Fat</td>
<td>11.3g</td>
<td>51.5g</td>
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<td>2.3g</td>
<td>10.3g</td>
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<tr>
<td>Carbohydrate</td>
<td>2.9g</td>
<td>13.2g</td>
<td></td>
</tr>
<tr>
<td>-Sugar</td>
<td>1.9g</td>
<td>8.5g</td>
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<tr>
<td>Sodium</td>
<td>127mg</td>
<td>576mg</td>
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<table>
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<th>Nutrition Information</th>
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</thead>
<tbody>
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<td></td>
</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Serving Size:</td>
<td>22g</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Quantity</strong></td>
<td><strong>Per Serving</strong></td>
<td><strong>Quantity</strong></td>
<td><strong>Per 100g</strong></td>
</tr>
<tr>
<td>Energy</td>
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<td>2330kJ</td>
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<tr>
<td>Protein</td>
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<td>17.4g</td>
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<tr>
<td>Total Fat</td>
<td>8.3g</td>
<td>37.6g</td>
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<td>7.7g</td>
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<tr>
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<tr>
<td>-Sugar</td>
<td>3.4g</td>
<td>15.4g</td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>136mg</td>
<td>617mg</td>
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**Hummus**

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<td>Quantity Per Serving</td>
<td>Quantity Per 100g</td>
</tr>
<tr>
<td>Energy</td>
<td>135kJ</td>
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<td>Protein</td>
<td>0.6g</td>
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<tr>
<td>Total Fat</td>
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</tr>
<tr>
<td>-Saturated</td>
<td>0.2g</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>1.0g</td>
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<tr>
<td>-Sugar</td>
<td>0.3g</td>
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<tr>
<td>Sodium</td>
<td>39mg</td>
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**Salad Dressing**

<table>
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<tr>
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<tr>
<td>Serving Size: 20mL</td>
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<tr>
<td>Quantity Per Serving</td>
<td>Quantity Per 100mL</td>
</tr>
<tr>
<td>Energy</td>
<td>186kJ</td>
</tr>
<tr>
<td>Protein</td>
<td>&lt;1.0g</td>
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<tr>
<td>Total Fat</td>
<td>4.0g</td>
</tr>
<tr>
<td>-Saturated</td>
<td>0g</td>
</tr>
<tr>
<td>Carbohydrate</td>
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<tr>
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<td>2.0g</td>
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<td>Sodium</td>
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## Processed Cheese

### Nutrition Information

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<td>Total Fat</td>
<td>3.8g</td>
</tr>
<tr>
<td>-Saturated</td>
<td>2.7g</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>&lt;1.0g</td>
</tr>
<tr>
<td>-Sugar</td>
<td>&lt;1.0g</td>
</tr>
<tr>
<td>Sodium</td>
<td>242mg</td>
</tr>
</tbody>
</table>

- Servings per package: 12
- Serving Size: 18g

### Nutrition Information

<table>
<thead>
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<th>Quantity Per Serving</th>
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</tr>
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<tr>
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<tr>
<td>Total Fat</td>
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<tr>
<td>-Saturated</td>
<td>&lt;1.0g</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>2.0g</td>
</tr>
<tr>
<td>-Sugar</td>
<td>1.9g</td>
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<tr>
<td>Sodium</td>
<td>234mg</td>
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</table>

- Servings per package: 20
- Serving Size: 18g

## Cream Cheese

### Nutrition Information

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<td>Protein</td>
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<td>Total Fat</td>
<td>6.1g</td>
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<tr>
<td>-Saturated</td>
<td>4.3g</td>
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<tr>
<td>Carbohydrate</td>
<td>&lt;1.0g</td>
</tr>
<tr>
<td>-Sugar</td>
<td>&lt;1.0g</td>
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<tr>
<td>Sodium</td>
<td>88mg</td>
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</table>

- Servings per package: 10
- Serving Size: 25g

### Nutrition Information

<table>
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<th>Quantity Per 100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
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<td>Protein</td>
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<tr>
<td>Total Fat</td>
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</tr>
<tr>
<td>-Saturated</td>
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</tr>
<tr>
<td>Carbohydrate</td>
<td>&lt;1.0g</td>
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<tr>
<td>-Sugar</td>
<td>&lt;1.0g</td>
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<tr>
<td>Sodium</td>
<td>66mg</td>
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- Servings per package: 10
- Serving Size: 25g
# Chocolate Mousse

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</tr>
<tr>
<td><strong>Quantity</strong></td>
<td><strong>Quantity</strong></td>
</tr>
<tr>
<td><strong>Per Serving</strong></td>
<td><strong>Per 100g</strong></td>
</tr>
<tr>
<td><strong>Per Serving</strong></td>
<td><strong>Per 100g</strong></td>
</tr>
<tr>
<td>Energy</td>
<td>543kJ</td>
</tr>
<tr>
<td>Protein</td>
<td>3.0g</td>
</tr>
<tr>
<td>Total Fat</td>
<td>7.3g</td>
</tr>
<tr>
<td>-Saturated</td>
<td>5.1g</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>12.9g</td>
</tr>
<tr>
<td>-Sugar</td>
<td>12.0g</td>
</tr>
<tr>
<td>Sodium</td>
<td>28mg</td>
</tr>
</tbody>
</table>

| Energy | 288kJ | 465kJ |
| Protein | 3.0g | 4.8g |
| Total Fat | 1.5g | 2.4g |
| -Saturated | 1.1g | 1.7g |
| Carbohydrate | 9.8g | 15.8g |
| -Sugar | 9.1g | 14.7g |
| Sodium | 44mg | 71mg |
### Appendix I – Five Prototypical Tastant Concentrations

<table>
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<th>Taste</th>
<th>Tastant</th>
<th>Sample Concentration</th>
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<td></td>
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<td>Weak</td>
</tr>
<tr>
<td>Sweet</td>
<td>Sucrose</td>
<td>100mM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13.69g/400mL</td>
</tr>
<tr>
<td>Salty</td>
<td>Sodium Chloride</td>
<td>100mM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.34g/400mL</td>
</tr>
<tr>
<td>Sour</td>
<td>Citric Acid</td>
<td>1mM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>77mg/400mL</td>
</tr>
<tr>
<td>Bitter</td>
<td>Caffeine</td>
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<tr>
<td></td>
<td></td>
<td>78mg/400mL</td>
</tr>
<tr>
<td>Umami</td>
<td>Monosodium Glutamate</td>
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<tr>
<td></td>
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<td>203mg/400mL</td>
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Appendix J – Labelled Magnitude Scale
Appendices

Appendix K – Papillae Stimulus Data Collection Form

Participant ID:

Testing Date:

Fat Taste Tongue Locations

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<th></th>
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<th>Foliate</th>
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<th></th>
<th>Circumvallate</th>
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<td></td>
<td>Paraffin</td>
<td>Oleic Acid</td>
<td>Paraffin</td>
<td>Oleic Acid</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>Trial 2</td>
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<td></td>
<td></td>
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<tr>
<td>Right</td>
<td>Trial 1</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Trial 2</td>
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</table>

Height _________

Weight _________

DOB _________
Appendices

Appendix L – Fat Taste Threshold (Fungiform) Data Collection Form

Instructions for Tasting Samples using Filter Paper

1. Pick up the filter paper samples from the end that is sticking out of the medicine cups. Do not touch the ‘wet’ end of the filter paper with your fingers.

2. Place the ‘wet’ end of the filter paper on the front part of your tongue (as pictured), with the liquid facing down onto your tongue.

3. Keep the filter paper on your tongue for 3 seconds, then place it back in the same cup it came from.

4. Do this for all 3 samples on your tray, then push the sample that tasted different to the back of your tray.

5. Press the ‘call’ button when you are ready to continue, and ensure you rinse your mouth out with water.

6. You will be given a 1 minute break between samples.
## Filter Paper Test

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<td></td>
<td></td>
<td></td>
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<td>1.8</td>
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<td>3.2</td>
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<td>5.7</td>
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<td></td>
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<tr>
<td>10</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>17.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31.9</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>50</td>
<td>Fat =</td>
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</table>
Appendix M – Example of Papillae Biopsy
Appendix N – Low-Fat Dietary Intervention Booklet

Following a low fat diet

Deakin University research study
About the study

Whilst participating in this study, we would like you to consume 15% of your total energy intake from fat on a daily basis. This is quite a small amount of fat as the average Australian normally consumes around 32% fat per day.

**15% fat = 25-30g***

* The dietitian will calculate your exact daily fat allowance

This diet is designed to reduce your fat intake, but is not a weight loss diet. Therefore, we will ask you to increase the amount of carbohydrate and protein you are currently eating to make sure that you do not lose weight during this study.

You will need to follow this diet for 8 **weeks**.
Your low fat suggested menu

Breakfast

__________________________________________________________________________
__________________________________________________________________________
__________________________________________________________________________

Morning tea

__________________________________________________________________________
__________________________________________________________________________
__________________________________________________________________________

Lunch

__________________________________________________________________________
__________________________________________________________________________
__________________________________________________________________________

Afternoon tea

__________________________________________________________________________
__________________________________________________________________________
__________________________________________________________________________

Dinner

__________________________________________________________________________
__________________________________________________________________________
__________________________________________________________________________

Dessert/ Supper

__________________________________________________________________________
__________________________________________________________________________
General instructions

1. You must NOT consume more than your daily allowance of meat provided to you in the previous list.
2. You must not exceed you daily allowance of fat.
3. Avoid eating any high fat foods (e.g. fried foods, fish and chips, regular fat salad dressing, cream, mayonnaise, cakes, pastries, biscuits, crisps, cheese).
4. All dairy products must be skim varieties and only ricotta and cottage cheeses are allowed (NO CHEDDAR or FULL FAT SOFT CHEESES (e.g. brie, camembert, blue etc.).
5. Avoid nuts or nutty spread (e.g. peanut butter and Nutella).
6. Lean cuts of red meat must be chosen and all fat must be trimmed from meat.
7. Eat as much as you like of fruits, vegetables, rice, lentils, beans, pasta, breads, cereals (with a couple of exceptions), skim milk, skim yogurt, ricotta cheese.
8. Any soft drinks, cordials and alcoholic can be included except for cocktails made with cream.
9. Boiled Lollies (e.g. minties, jellies etc.) can be included but avoid chocolate or toffees.
Fats and oils

No more than the recommended servings of reduced fat butter, reduced fat margarine, avocado, olive oil, canola oil.

Use oil and butter sparingly (try cooking with spray oil). Avoid butter, deep frying and cooking with large quantities of fat.

Meats

Lean mince (95% fat free or 5g fat/100g), lean steaks, chicken fillets without skin, veal, kangaroo, fish (no batter), seafood cooked by low fat method (grill, poach or bake), tuna in spring water or 98% fat-free flavoured tuna, lean sandwich meats (chicken, turkey or ham)

Regular fat mince, bacon, sausages, ribs, salami, fried chicken, chicken nuggets, chicken kiev, crumbed/battered fish, fish canned in oil, deli meats, duck, offal, pork belly, pork crackling, and fatty fish e.g. salmon
Cheese

**GO** INCLUDE

- Low fat ricotta and cottage cheese (95% fat free)

**STOP** AVOID

- Cheddar, all soft cheeses (e.g. brie, camembert, blue, feta, mascarpone, parmesan), full-fat hard cheeses and cream cheese.

Breads and cereals

**GO** INCLUDE

- Plain breads including white, wholemeal, wholegrain, rye.
- Breakfast cereals, boiled pasta, low fat noodles, rice, ryvita, light premiums/salads, cruskits, rice cakes

**STOP** AVOID

- Cereals containing coconut, seeds or nuts, toasted cereals, muesli, granola.
- Cheese rolls & pizza rolls from bakeries, croissants, donuts, muffins, pastries, muesli bars, croissants.
Appendices

Vegetables and fruit

All vegetables cooked without fat and not fried. All fruit without fat added

Frozen potato chips/ gems, roast vegetables cooked with oil, coleslaw, potato salad, fruit pies/ crumbles, coleslaw, potato salad

Condiments

Sugar, honey, jam, pepper, curry powder, mustard, spices, herbs, vinegar, ginger, apple or cranberry sauce, mint sauce, chilli sauce, golden syrup, maple syrup, low fat salad dressing, salt, Worcestershire sauce, soy sauce, pickles, vegemite, fish sauce

Limit mayonnaise, salad dressings, gravies and sauces, unless they are homemade with low-fat ingredients.
Avoid: Peanut butter, hazelnut spreads (e.g. Nutella), tahini, mayonnaise, regular fat salad dressing, olives, dips (e.g. French onion, guacamole, pesto etc.)
Desserts and cakes

**GO**
- Jelly, sorbet, fruit salad, reduced fat ice-cream (95% fat free), reduced fat custard (99% fat free), boiled lollies, jelly beans, jams, marmalades, unbuttered popcorn.

**INCLUDE**

**STOP**
- All cakes, pastries, biscuits, cookies, puddings, ice-cream, full fat custard, cream, chocolate or caramels, potato crisps, corn chips, buttered popcorn.

**AVOID**

Milk/ yoghurt

**GO**
- Skim (non-fat) milk, skim yoghurt (plain or fruit flavoured)

**INCLUDE**

**STOP**
- Full fat; milk, yoghurt, cream, sour cream, condensed milk, ice-cream, custard and sauces made with

Eggs

**GO**
- Egg whites and poached or boiled scrambled eggs (up to a maximum of 2 eggs/day). Prepared without added fat or full cream milk.

**INCLUDE**

**STOP**
- Fried eggs. Eating out; scrambled eggs or omelette.
**Beverages**

**GO INCLUDE**

- Tea, coffee, water, fruit juice, soft drink, mineral water, cordial, beer, wine, spirits, Milo (made with water and skim milk), sports drinks.

**STOP AVOID**

- Drinks made with full fat milk (e.g. take away coffees and hot chocolates, milk shakes, iced coffee/ chocs), cocktails made with cream

**Snack foods**

**GO INCLUDE**

- Rice crackers, rice cakes, corn thins, popcorn with no butter, pita bread with low fat tzatziki (97% fat free/ 3g fat/ 100g), dried fruit, and canned fruit.

**STOP AVOID**

- Potato chips, savoury biscuits (e.g. Savoys, Shapes, Salada), sweet biscuits (e.g. Tim tams, scotch fingers etc.), buttered popcorn, all nuts

**Sweets and lollies**

**GO INCLUDE**

- Boiled sweets, jubes, barley sugar, liquorice etc.

**STOP AVOID**

- All chocolate and caramels/toffees
Take away foods

**GO**
- Grilled fish, sliced roast meat (no fat, no gravy), salads (no dressings), sandwiches/ wraps, tomato-based pasta dishes (no cheese), tomato based soups, sushi, sashimi
- Subway (6 Inch Sub with Less than 6g of Fat range)
- Baked Potato (no butter, sour cream, cheese, bacon, ham)

**STOP**
- Most take away foods including battered fish and chips, pizza, fried and BBQ chicken, hot chips, hamburgers, pizza, Thai, Indian, Chinese etc., any other fried foods (e.g. spring rolls, dim sims, chicken strips, chicken schnitzel burgers etc.)

**INCLUDE**
- Take away foods

**AVOID**
Recommended foods and brands

Purchase the following at the supermarket:

Fresh fruit
Vegetables
Olive or canola oil
Margarine and reduced fat margarine (20-70% fat)
Chicken breast, fish and lean red meat
Milk - no fat (99.9% fat free or less than 1g fat/ 100g) or low fat (98% fat free or less than 2g fat/ 100g)
Yoghurt - no fat (99% fat free or less than 1g fat/ 100g) or low fat (98% fat free or 2g fat/ 100g)
Cheese - low fat (less than 8g fat/ 100g)
Legumes (chickpeas, beans and lentils)
Corn thins
Plan, low fat rice crackers
Dried fruits
Lollies
Breads and cereals

The following list gives examples of low fat products but many more are available – check the labels on products

Fats and oils
Margarine
Flora, Coles, Meadow Lea, Nuttelex, Olive Grove etc.
Reduced fat margarines
Weight Watchers margarine canola tub (60% fat)
Flora Pro-Activ ultra-light (30 and 50% fat varieties)
Flora extra light (30% fat)
Meadow Lea canola extra light (30% fat)
Nuttelex light (50% fat)
Milk and milk products

Skim milk
Physical no fat milk
Pura Tone no fat milk
Coles skim milk
Skim yoghurts
Yoplait plain/fruit no fat yoghurt
Nestle diet plain/fruit no fat yoghurt
Danone Oikos plain/fruit Greek yoghurt
Chobani plain/fruit Greek yoghurt

Cheeses

Reduced fat ricotta
Pantalica light ricotta cheese
Perfect Italian Smooth light ricotta cheese
fresh low fat ricotta cheese from the deli
Reduced fat cottage
Bulla Low Fat
Weight Watchers
Farmland Low Fat

Cereals / breads

Wholemeal or multi grain bread/rolls
Wholegrain cereals
Oats (Uncle Toby’s, Lowans)
Weetbix
Kellogg's Mini-wheats
Sanitarium Lite Bix
Special K

untoasted natural muesli without nuts

Coles low fat muesli
Pure Harvest organic natural muesli
Sun Sol fruity muesli

**Dressing**

- Vinegar (balsamic, white, red wine etc.)
- Lemon juice
- Commercial salad dressings
  - Coles Simply Less varieties (e.g. Italian, balsamic, French, honey mustard etc.)
  - Kraft fat free varieties (e.g. French, coleslaw, Italian, balsamic etc.)

**Snacks**

- Rice crackers (Sakata, Coles, Fantastic, Vita-Weat, Sunrice Japanese)
- Rice cakes (Sunrice, Coles Simply Less)
- Corn Thins (Real Foods corn thins, Sunrice flavoured rice cakes)
- Popcorn (Sunbites air-popped popcorn)
Cooking ideas at home

**Meat**
Marinate skinless chicken breast fillets or lean meats in soy sauce, lemon, or herbs and spices before grilling
Bake fish fillets or kebabs in foil with seasonings and lemon juice
Make kebabs using lean meat (trim any excess fat) and vegetable chunks (see recipe)

**Cooking vegetables**
Add a squeeze of lemon or ground black pepper to freshly steamed or microwaved vegetables for flavour
Stir fry vegetables using water or stock
Add natural, fat-free yoghurt to baked potatoes instead of sour cream
Pre-cook whole potatoes in the microwave or oven, then crisp on the BBQ

**Dinner**
Use low fat ricotta cheese in cheese dishes

**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

**Pasta**
Serve pasta with a tomato based sauce
Avoid creamy and cheese based pasta sauce

**Toast**
Spread with ricotta or cottage cheese, marmalade, honey, chutney, mustard or top with a small serve of baked beans, spaghetti or fresh tomato

**Roast Dinner**
Select a lean leg of lamb, beef, or pork – trim off any fat, cook on a baking grid in the roasting dish so that roast is not cooking in fat
Avoid skin on roast a chicken
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
When eating out

Modify meals to suit you

Check the ingredients and preparation method of meals with your waiter

Ask to replace high-fat ingredients with low-fat options. For example: vegetables instead of chips, skim milk rather than full cream

Request sauces or dressings on the side, that way you can control the quantity on your plate

Don’t being swayed by pushy waiters or jibes from your fellow dinners; just order what you want

Know what is good and what to avoid

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 fish have less fat than the leanest meats

Avoid:

Creamy soups, stick to clear ones instead

Fatty steak cuts such as porterhouse and ribs

Quiches and omelettes if made with cheese

Antipastos
**Breakfast**

Opt for untoasted muesli with low fat yoghurt and cereals.

Most muffins are high in fat. Avoid these where possible.

English muffins and bagels are good choices, but ask for spreads such as jam or vegemite on the side.

Always ask for skinny milk in coffees, tea and milkshakes.

**Lunch and dinner**

Some salads can be made with heavy creamy dressings.

Choose pasta made with a tomato based sauce and avoid parmesan cheese.

Fill sandwiches with lots of salad vegetables and a small serving of lean meat, skinless chicken, canned tuna (in spring water), low fat 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.

**Dessert**

Fruit is your best option. Sorbets, yoghurts and fat reduced ice-cream are also good.
Useful tips and points to remember

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 e.g. basil, thyme, paprika, cumin, bay leaves and different spice mixes

Use the provided check list to keep track of the amount of fats and oils you are consuming
Low fat recipes
Grilled capsicum and zucchini pizza with feta

Serves: 2  
Preparation time: 5 mins  
Cooking time: 5 mins

Ingredients

- 2 medium-sized wholemeal pita breads (23cm in diameter)
- 1 garlic clove, halved
- Spray oil
- 100g char-grilled zucchini strips
- 50g cherry tomatoes, halved
- 50g low-fat feta, crumbled
- 1/4 teaspoon dried oregano
- Freshly ground black pepper
- 4 handfuls rocket, to serve

Method

1. Preheat the grill to hot. Place the pita breads under the grill for 1 minute until crisp, then remove from the grill and rub the grilled side with the garlic. Turn each pita over and spray with olive oil.
2. Top with capsicum, zucchini, tomato (cut-side up) and low fat feta. Sprinkle with oregano and season with pepper.
3. Place under the grill for 2 minutes until the cheese lightly browns. Serve with the rocket.
Minestrone soup

Recipe source: Australian Good Taste - July 2002, Page 94
Preparation time: 20 mins
Cooking time: 1 hour
Serves: 6

Ingredients

- 400g can diced peeled tomatoes
- 3 pontiac potatoes, peeled, chopped
- 200g piece jap or butternut pumpkin, deseeded, peeled, chopped
- 1 large brown onion, chopped
- 2 carrots, chopped
- 2 celery sticks with leaves, chopped
- 1 large garlic clove, chopped
- 1 tsp dried oregano leaves
- 1.75L (7 cups) water
- 3 small or 2 large zucchini, chopped
- 40g (1/4 cup) small macaroni
- 1 400g can borlotti beans, rinsed, drained
- 1/2 cup firmly packed roughly chopped fresh continental parsley
- Salt & freshly ground black pepper

Method

1. Place the tomatoes, potatoes, pumpkin, onion, carrots, celery, garlic and oregano in a large saucepan.

2. Stir in the water and bring to the boil over medium-high heat. Reduce heat to medium and cook, almost covered, for 45 minutes.

3. Add the zucchini and macaroni, and cook, stirring occasionally, for 10 minutes. Stir in the beans and cook for a further 5 minutes or until the zucchini and pasta are tender.

4. Stir in the parsley and taste and season with salt and pepper. Ladle into serving bowls.
Brown lentil and vegetable soup

Recipe source: Super Food Ideas - July 2006, Page 32
Preparation time: 15 mins
Cooking time: 30 mins
Serves: 5

Ingredients

- Spray olive oil
- 2 carrots, peeled, diced
- 2 zucchini, diced
- 2 sticks celery, diced
- 1 brown onion, finely chopped
- 400g can diced tomatoes
- 2 cups salt-reduced vegetable stock
- 400g can brown lentils, rinsed, drained
- 1/2 cup flat-leaf parsley leaves, chopped
- 1 lemon, quartered
- 4 crusty wholegrain rolls, to serve

Method

1. Heat spray oil in a large saucepan over medium-high heat. Add carrots, zucchini, celery and onion. Cook, stirring occasionally, for 10 minutes or until vegetables begin to soften.

2. Add tomatoes and stock to pan. Cover and bring to the boil. Reduce heat to medium-low. Simmer, partially covered, for 15 minutes or until vegetables are soft.

3. Add lentils to soup. Cook for 5 minutes or until heated through. Ladle soup into bowls. Sprinkle with parsley and squeeze lemon juice. Season with freshly ground black pepper. Serve with lemon wedges and bread rolls.
Grilled fish with chickpea salad

Recipe source: Super Food Ideas - November 2006, Page 62
Preparation time: 15 mins
Cooking time: 15 mins
Serves: 5

Ingredients

- 300g orange sweet potato, peeled, chopped into 2cm cubes
- 1/2 teaspoon ground cumin
- 1 garlic clove, crushed
- Olive oil cooking spray
- 250g cherry tomatoes
- 600g firm white fish fillets (such as snapper or barramundi)
- 40g low-fat feta cheese
- 1/2 cup canned chickpeas, drained, rinsed
- 100g baby spinach

Yoghurt dressing

- 1/2 cup low-fat natural yoghurt
- 1/2 lemon, juiced
- 1 small garlic clove, crushed
- 2 tablespoons coriander leaves, finely chopped

Method

1. Preheat oven to 200°C. Combine sweet potato, cumin and garlic in a roasting pan. Season with salt and pepper. Spray with oil. Roast for 30 minutes. Add tomatoes. Roast for a further 15-20 mins or until sweet potato is tender. Set aside.

2. Preheat barbecue plate on high heat. Season fish with salt and pepper. Spray lightly with oil. Barbecue for 4 to 5 minutes each side or until just cooked through.

Miso-glazed fish with sesame brown rice salad

Recipe source: Australian Good Taste - September 2012, Page 50
Preparation time: 35 mins
Cooking time: 10 mins
Serves: 4

Ingredients

- 3 tsp miso paste
- 3 tsp salt-reduced soy sauce
- 2 tbs orange juice
- 2 garlic cloves, crushed
- 2 tsp honey
- 4 (about 150g each) firm white fish fillets
- 2 Lebanese cucumbers, trimmed
- 1 firm ripe avocado, peeled, cut into 1.5cm pieces
- 1 tbs rice vinegar
- 2 cups steamed brown rice
- 2 tsp sesame seeds, lightly toasted
- Steamed snow peas, to serve

Method

1. Combine the miso paste, soy sauce, orange juice, garlic and honey in a glass or ceramic bowl. Add the fish and turn to coat. Cover and place in the fridge for 30 minutes to marinate.

2. Preheat oven to 200C. Line a large baking tray with baking paper. Place a wire rack on top. Drain the fish from the marinade and place on the rack. Bake for 12 minutes or until the fish is golden and flakes easily when tested with a fork.

3. Meanwhile, use a vegetable peeler to peel the cucumber into ribbons. Place the cucumber and avocado in a bowl. Add the vinegar and toss to combine.

4. Divide the rice among plates. Top with the avocado mixture and fish. Sprinkle with the sesame seeds. Serve with steamed snow peas.
Chicken rice paper rolls

Recipe source: Australian Good Taste - October 2010, Page 26
Preparation: 20 mins
Cooking time: 5 mins
Serves: 16 rolls

Ingredients

- 1.25L (5 cups) water
- 1 brown onion, halved
- 1 tsp black peppercorns
- 2 single chicken breast fillets, no skin
- 8 rice paper sheets
- 1/2 cup mint leaves
- 100g snow pea sprouts, trimmed
- 8 baby cos lettuce leaves
- Vietnamese dipping sauce, to serve

Method


2. Shred chicken. Dip 1 rice paper sheet into warm water for 30 seconds or until it softens. Arrange chicken along one edge, leaving a 2cm border. Top with mint, snow pea sprouts and a lettuce leaf. Roll up to enclose. Halve.

3. Repeat with the remaining rice paper sheets, chicken, mint, snow pea sprouts and lettuce. Serve with sauce for dipping.
Chilli and lemongrass beef

Recipe source: Taste.com.au - September 2011
Preparation time: 20 mins
Cooking time: 15 mins
Serves: 4

Ingredients

- 1 long red chilli, chopped
- 1 lemongrass stem (inner core only), finely chopped
- 3cm piece ginger, chopped
- 3 garlic cloves
- 1 tbsp grated palm sugar
- Juice of 1 lime
- 3 tsp fish sauce
- Spray oil
- 500g rump steak, trimmed, thinly sliced
- 100g vermicelli noodles
- 2 spring onions, thinly sliced
- 1/2 cup each coriander, basil and mint leaves

Method

1. Place chilli, lemongrass, ginger and garlic in a food processor and whiz into a paste. Add palm sugar, lime juice, fish sauce and whiz to combine. Toss the beef in a bowl with half the marinade. Chill for 15 minutes.

2. Meanwhile, cook the vermicelli according to the packet instructions. Drain, refresh under cold water and toss with remaining marinade.

3. Spray provided oil in a wok or large pan over medium-high heat. Add half the beef and cook, turning, for 5-7 minutes, or until browned. Repeat with remaining 2 teaspoons oil and beef. Keep warm.

4. Toss spring onions and herbs with the rice noodles, then top with the beef and serve.
Low-fat moussaka rolls

Recipe source: Super Food Ideas - October 2011, Page 24  
Cooking time: 1 hour  
Serves: 4

Ingredients

- 1 large (800g) eggplant, cut lengthways into 5mm-thick slices  
- Olive oil cooking spray  
- 2 teaspoons lemon juice  
- 350g fresh reduced-fat ricotta cheese  
- 1 green onion, thinly sliced  
- 1 garlic clove, crushed  
- 1/4 cup flat-leaf parsley leaves, chopped  
- 1 teaspoon fresh oregano leaves, chopped  
- 1/4 teaspoon ground nutmeg  
- 1 1/2 cups tomato passata  
- 1/2 cup grated reduced-fat cheddar cheese  
- Salad leaves, to serve

Method

1. Preheat oven to 180°C/160°C fan-forced. Lightly spray eggplant with oil. Season with salt and pepper. Heat a frying pan over medium-high heat. Cook eggplant, in batches, for 3 minutes on each side or until golden. Transfer to a baking tray. Drizzle eggplant with lemon juice. Reserve 12 slices of eggplant. Chop remaining eggplant.

2. Combine ricotta, chopped eggplant, onion, garlic, parsley, oregano and nutmeg in a bowl. Season with salt and pepper. Place 1 eggplant slice on a flat surface. Top with 2 tablespoons of ricotta mixture. Roll up firmly to enclose filling. Place, seam-side down, in prepared dish. Repeat with remaining eggplant slices and ricotta.

3. Pour passata over eggplant rolls. Sprinkle with cheese. Bake for 30 to 35 minutes or until golden. Stand for 5 minutes before serving.
Balsamic chicken and white bean salad

Recipe source: Super Food Ideas - December 2005, Page 38
Preparation: 10mins
Cooking: 20 mins
Serves: 6

Ingredients

- 600g skinless chicken breast, all fat removed
- 2 garlic cloves, crushed
- 1 tablespoon wholegrain mustard
- 2 tablespoons balsamic vinegar
- Olive oil cooking spray
- 400g can cannellini beans, drained
- 250g cherry tomatoes, halved
- 1/4 cup low-fat feta cheese, crumbled
- 50g rocket leaves
- 1 lemon, cut into wedges, to serve

Method

1. Season chicken with salt and pepper. Whisk garlic, mustard and vinegar in a ceramic dish. Add chicken and turn to coat. Cover and refrigerate for at least 20 minutes.

2. Preheat a barbecue plate on high heat. Remove chicken from marinade. Lightly spray chicken with oil. Cook for 1 minute each side or until golden. Reduce heat to medium-low. Cook chicken for 6 to 8 minutes each side or until cooked through. Transfer to a plate. Cover and stand for 5 minutes.

3. Slice chicken. Combine beans, tomato, feta, rocket and chicken in a large bowl. Toss
Honey pumpkin penne

Recipe source: Adapted from Australian Good Taste - August 2012 Page 27
Preparation: 10mins
Cooking: 20 mins
Serves: 6

Ingredients

- 700g Kent pumpkin, peeled, cut into 2cm pieces
- Spray olive oil
- 1 tbs red wine vinegar
- 2 tsp honey
- 300g dried penne pasta
- 100g fresh low-fat ricotta, crumbled
- 2 spring onions (shallots), thinly sliced
- 2 tbs chopped fresh continental parsley
- 1 cup baby spinach
- 1 tbs chopped fresh basil

Method

1. Preheat oven to 220°C. Place the pumpkin on a baking tray and spray with olive oil. Combine the vinegar and honey in a small bowl. Season. Pour half the mixture over the pumpkin. Toss to combine. Roast, stirring halfway through cooking, for 20 minutes or until golden and tender.

2. Meanwhile, cook the pasta in a large saucepan of salted boiling water following packet directions or until al dente. Refresh under cold water. Drain.

3. Place the pasta, pumpkin, ricotta, spring onion, parsley, spinach and basil in a large bowl. Pour over remaining mixture vinegar and honey mixture and season. Toss gently to combine.
Mushroom, zucchini and fresh tomato pasta

Preparation time: 10 mins
Cooking time: 10 mins
Serves: 2

Ingredients

- 160g pasta
- Spray olive oil
- 1 clove garlic
- 1 medium zucchini
- 1 ½ teaspoon dried oregano leaves
- 12 cherry tomatoes
- Pinch sea salt
- ¾ cup fresh basil leaves
- 60g low-fat ricotta

Method

1. Cook pasta in a large saucepan of boiling water according to the packet instructions or until al dente. Drain.

2. Heat a large non-stick frying pan over medium heat and spray with oil. Add garlic, zucchini, mushrooms and 1 tsp oregano; cook for 2 minutes.

3. Add tomatoes, salt and 1/3 cup basil; cook for a further 2 minutes. Add the pasta to the pan and mix well; cook for an additional minute. If the mixture looks dry, add a few tablespoons of water to moisten it.

4. Transfer pasta to serving bowls and crumble with ricotta, oregano and remaining basil. The below picture used parmesan cheese, however this is too high in fat for your diet plan.
Spice-crusted lamb cutlets

Recipe source: Australian Good Taste - April 2005, Page 119
Preparation time: 10 mins
Cooking time: 5 mins
Serves: 8

Ingredients

- 16 (about 700g) lamb cutlets, **excess fat trimmed**
- 3 garlic cloves, crushed
- 2 tbs fresh lemon juice
- 3 tsp honey
- 2 tsp finely grated fresh ginger
- 2 tsp ground cumin
- 1 tsp ground coriander
- 1/2 tsp ground cinnamon
- 1 cup steamed vegetables to serve

Method

1. Place the lamb cutlets in a large glass or ceramic dish. Combine the garlic, lemon juice, honey, ginger, cumin, ground coriander and cinnamon in a small bowl.

2. Pour the marinade over the lamb and rub to evenly coat. Cover with plastic wrap and place in the fridge for 2 hours to develop the flavours.

3. Preheat a barbecue grill or chargrill on high. Reduce heat to medium. Add the lamb and cook for 2 minutes each side for medium-rare or until cooked to your liking.

4. Transfer lamb cutlets to a serving platter. Cover with foil and set aside for 5 minutes to rest.

5. Serve spice-crusted lamb cutlets with the pasta salad.
Corn fritters

Ingredients

- 1 tin (400g) corn kernels
- 1 medium zucchini, grated and liquid drained
- 2 spring onions, sliced
- ¼ cup plain flour
- 1 egg
- Salt and pepper
- Spray olive oil

Salad

- ½ punnet cherry tomatoes, quartered
- ½ spinach
- ½ cup rocket
- ½ Lebanese cucumber, sliced
- Lemon juice

To serve

Yoghurt dressing

- ¼ cup natural Greek low fat yoghurt
- 3tbs lemon juice
- 2 tbs fresh mint

Method

1. Place the corn kernels, zucchini, spring onions, flour, egg, salt and paper in a bowl and combine.

2. Spray pan lightly with olive oil spray and heat on medium to high. Spoon heaped tablespoons of the mixture and fry on one side until golden. Flip and fry other side. Serve with 2tbs yoghurt dressing, salad and a squeeze of lemon juice.
Herb and garlic beef kebabs

Ingredients

- You'll need 8 skewers for this recipe
- 700g rump steak, fat trimmed, cut into 1cm thick strips
- 3 cloves garlic, crushed
- 1½ teaspoons dried mixed herbs
- 1 teaspoon olive oil
- 8 chat potatoes
- 1½ tablespoons lemon juice
- 1 teaspoon Dijon mustard
- Pinch of caster sugar
- 1 red capsicum, deseeded, diced
- ½ red onion, diced
- 80g baby rocket leaves

Method

1. Soak skewers in a shallow dish of cold water for 30 minutes. Drain.

2. Place beef into a bowl with garlic, herbs and 1 teaspoon oil, and salt and pepper. Thread onto to skewers. Place onto plate. Cover. Refrigerate for 1 to 2 hours, if time permits.

3. Meanwhile, cook potatoes in a saucepan of boiling salted water for 15 minutes or until just tender. Drain. Thickly slice.

4. Combine lemon juice, mustard, sugar, and salt and pepper into a screw top jar. Shake well. Place potatoes in a bowl with capsicum, onion, rocket and dressing. Toss to combine.

5. Preheat a barbeque grill on high heat until hot. Reduce heat to medium. Cook skewers, turning, for 4 minutes for medium.
Appendix O – High-Fat Dietary Intervention Booklet

Deakin University research study

Following a high fat diet
Your high fat menu sample

Breakfast

Morning tea

Lunch

Afternoon tea

Dinner

Dessert/ Supper
General instructions

1. You must consume your daily allowance of fat provided to you in the previous list
2. All dairy products must be full-fat varieties
3. Avoid ‘light’ or ‘reduced fat’ products
4. Feel free to add condiments (e.g. salad dressing, sour cream, mayonnaise etc.)
5. Consume plenty of nuts and nutty spreads (e.g. peanut butter)
Foods to maintain healthy fat intake

Increase your consumption of the following

- Added fats e.g. butter, margarine, olive oil, other oils
- Full-fat milk
- Full-fat yoghurt
- All types of cheese
- Nuts and nut spreads
- Avocado
- Olives
- Dark chocolate
- Red meat
- Fish
- Eggs

BUT...

You can consume anything you feel like whilst following this diet as long as you consume your daily allowances (8 teaspoons) of fats per day.
How to add more fat to your diet

- Add butter/margarine and cream to your mashed potato
- Choose fried or battered foods
- Put butter/margarine, or grated cheese on your vegetables
- Cook all meats in oil
- Use salad dressing made with oil or mayonnaise on salads and sandwiches
- Bake or fry in olive/canola/sunflower oil
- Use creamy white sauce or gravy with meals
- Spread butter/margarine, avocado or peanut butter generously over bread or toast
- Stir cream into soups
- Add cream, custard, ice cream to desserts
- Add cream to scones, pikelets and cakes
- Snack on chips, biscuits and dips, cheese and biscuits, chocolate, nuts, biscuits, muffins, cake, croissants etc.
- Make a milkshake as a snack with full-fat milk and ice cream, or have a flavoured milk drink (e.g. Big M, Classic, Breaka) with a meal
- Choose quiches, pasties, pies and sausage rolls as lunches or dinners
Breakfast Ideas;

- Fried eggs or an omelette
- Eggs Florentine/Benedict
- Sausages or sausage patties
- Pancakes made with FF milk & butter
- Waffles
- Bagels with cream cheese
- French toast made with butter
- Toast with peanut butter, avocado and/or cheese
- Raisin toast or crumpets with butter/margarine
- Scrambled eggs made with FF milk, margarine/butter and cheese - you can also include tomatoes, mushroom, bacon and/or spinach.

- Scones with cream & jam
- Croissant with butter & jam
- Croissant with ham & cheese
- Banana smoothie with ice-cream & peanut butter
- Avocado smoothie (see recipes in diet booklet)
- FF yoghurt & muesli
- Toasted muesli & FF milk
- Smashed avocado (avocado & fetta) on toast with buttered mushrooms

FF = Full fat

*Remember to avoid light or reduced fat products.*
Lunch and Dinner Ideas;

- Ham & cheese omelette
- Fried Pork chops with mashed potato with added butter
- Creamy soups
- Fried chicken or veal schnitzel
- Nachos with guacamole & sour cream
- Cheese burger with egg
- Chicken wings
- Creamy curries
- Creamy pasta dishes
- Cheesy tuna casserole
- Chicken & cashew noodle stir-fry
- Fried/crumbed fish Cauliflower cheese sauce made with full fat milk
- Smoked salmon & cream cheese roll-ups
- Fried Fish cakes
- Deep-fried Chicken
- High fat Minced beef tacos/burrito
- Fried Sausages, Ham/salami plus cheese plus avocado/ roll
- Peanut butter roll
- Pasties/pies/sausage rolls
- Quiche - basic recipe; eggs, heavy cream, onion, butter and cheese, plus seasoning.
  You can then vary the flavour by adding more vegetables, different cheeses or meat

Tips

* Bake or fry in olive/canola/sunflower oil
* Use creamy white sauce (made with full fat milk and/or cream or gravy made with fat from meat with meals
* Add butter/ margarine and cream to your mashed potato
* Put butter/ margarine or grated cheese on your vegetables
* Use salad dressing made with oil or mayonnaise on salads and sandwiches
Appendices

Snack Ideas;

- 1/2 Avocado
- Cheese and crackers
- Nuts e.g. almonds, peanuts, cashews, pecans, walnuts,
- Seeds e.g. sunflower, pumpkin
- Nutella/ peanut butter on crackers
- Tuna in oil
- Salmon in oil
- Full Fat yoghurt
- Full cream milk shake made with ice-cream
- Big M
- Olives/ stuffed olives
- Pepperoni/ salami
- coleslaw or potato salad made with oil/egg based mayonnaise
- Chocolate
- Hard boiled eggs/ devilled eggs
- Soft boiled eggs with buttered toast soldiers
- Instant puddings make with full cream milk and added cream
- Muesli bars, particularly those with nuts and/or chocolate
- High fat biscuits such as shortbread and chocolate biscuits
- Cheesecake
- Smoked salmon with cream cheese
- Dips (ranch, hummus, pesto etc.) with high fat potato crisps or high fat savoury biscuits
- High fat high fat potato crisps or high fat savoury biscuits with peanut butter or cream cheese
- Guacamole and fried corn chips
Recipes
Ham and Cauliflower Casserole au Gratin
Serves 6

**Ingredients:**
6 cups cauliflower flowerets, cut fairly small
2 cups diced ham/bacon
1/2 cream cheese, softened
3/4 cup Greek yogurt
1/4 finely-grated Parmesan cheese
1/4 cup thinly sliced green onions
3/4 cup grated cheese

**Instructions:**

1. Preheat oven to 350F/180C. Fill a medium-sized pot half full with water and start to bring to a boil. Cut up the cauliflower into small flowerets, discarding the leaves and core. Put cauliflower pieces into the water and cook at a low boil until the cauliflower is starting to get soft, about 20 minutes. (It doesn’t need to be completely cooked, but it should be soft enough to mash.)

2. While cauliflower is cooking, cut the ham/bacon into small cubes and slice green onions. Put cream cheese into a dish and soften it in the microwave for a few minutes. When cream cheese is soft stir in the Greek yogurt, Parmesan, and sliced green onions.

3. When cauliflower is cooked enough to start to soften, pour into a colander placed in the sink and let it drain well (at least 5 minutes.) Put the drained cauliflower back into the pot you cooked it in and mash with a potato masher until it’s partly mashed but still has quite a few chunks left. Stir in the sauce mixture and combine well with the cauliflower; then gently mix in the diced ham.

4. Bake 30-35 minutes, or until the cheese is melted and lightly browned and all the mixture is hot throughout and starting to bubble. Let sit 10 minutes for any liquid to be absorbed, then serve hot.
Cobb Salad
Makes 2 portions

Ingredients for the dressing:
- 3 tbs of olive oil
- 2 tbs apple cider vinegar
- 1 tsp of lemon juice
- 1 tsp of Dijon Mustard
- 1 clove of garlic (optional)
- Salt and pepper to taste

Ingredients for the salad:
- 2 tbs Olive Oil
- 100 grams of ham or bacon
- 4 cherry tomatoes
- 30 grams of blue cheese
- 2 hard-boiled eggs
- 2 cups of lettuce coarsely chopped
- ½ avocado diced
- 1 chicken fillet with skin on or chicken schnitzel

Directions:
1. Hard boil the eggs
2. Cook the chicken in olive oil
3. Slice the ham in cubes and heat in a skillet sprayed with olive oil for 3-5 minutes
4. Slice the hard-boiled eggs
5. Put the lettuce in the bottom of the bowl
6. Put the salad ingredients in rows next to each other like shown in the picture
7. Evenly spread the dressing over the salad
Basic Cheese and Onion Quiche

Makes 2 quiches

**Ingredients:**
- 2 sheets shortcrust pastry
- 5-6 cups shredded cheese, divided in half
- 2 tbs butter plus more for greasing pans
- 1 large white onion, finely chopped
- 12 large eggs
- 2 cups heavy cream
- 1 tsp salt
- 1 tsp ground black pepper
- 2 tsp dried thyme

**Directions:**

1. Preheat oven to 350 degrees

2. Lightly press the pastry into two 10 inch quiche pans or deep pie pans. Blind-bake the pastry in the oven for 10 minutes.

3. In a skillet, add the butter and melt over medium heat. Then add the vegetables and saute until onions are translucent and soft. Remove from heat and cool.

4. Put 2 cups of shredded cheese in bottom of each of the pastry shells. Add 1/2 of cooled vegetable mixture to each pan in an even layer over cheese.

5. Crack 12 eggs and pour into a large mixing bowl. Add the cream and spices, and whisk together until well mixed and frothy. Pour 1/2 mixture over each pan of cheese and veggies, then use a fork to gently and evenly distribute cheese and vegetables into egg and cream mixture.

6. Slide quiche pans into oven, leaving an inch of space between pans. Bake for about 20-25 minutes or until set and puffy and slightly golden in the center.
Cottage Pie
Serves 4

Ingredients:
1 tbs olive oil
1 medium brown onion, chopped
600g beef mince at least 15% fat
1 large carrot, peeled, finely chopped
1 medium zucchini, finely chopped
3 celery stalks, trimmed, finely chopped
2 tbs tomato paste
400g can chopped tomatoes
1/2 cup beef stock
850g sebago potatoes, peeled, chopped
1/4 cup full cream milk
20g butter
1/2 cup grated tasty cheese
Mixed salad leaves, to serve

Directions:

2. Add carrot, zucchini and celery. Cook, stirring, for 3 minutes. Add tomato paste, tomatoes and stock, and stir. Reduce heat to medium-low. Cover. Simmer for 15 minutes or until vegetables are tender. Preheat oven to 200°C/180°C fan-forced.


4. Spoon mince mixture into four 1 3/4 cup capacity ovenproof dishes. Top with mash. Sprinkle with cheese. Bake for 15 to 20 minutes or until golden.
Fish Burger
Serves 4

Ingredients:
4 frozen crumbed fish fillets
Olive oil for cooking
2 small Lebanese cucumbers
8 butter lettuce leaves
1 avocado, sliced
4 long white bread rolls, split, toasted
Butter/margarine
1/3 cup whole-egg aioli

Directions:
1. Fry fish using olive oil
2. Using a vegetable peeler, peel cucumbers into ribbons.
3. Divide lettuce and avocado between roll bases.
Pistachio-crusted Salmon
Serves 8

Ingredients:
175g packet pistachios, finely chopped
1/2 bunch dill, chopped
1 lemon, finely grated and zest
8 small salmon fillets (about 180g each)
Olive oil spray

Directions:
1. Preheat oven to 190C or 170C fan-forced and line a large baking tray with non-stick baking paper. Combine pistachios, dill and lemon zest in a bowl.
2. Press top side of each salmon fillet into the pistachio mixture to coat evenly. Sprinkle any leftover mixture on fillets. Drizzle generous amounts of olive oil over the fish fillets.
3. Bake salmon for 5 minutes, then cover loosely with foil and cook a further 5 minutes. Uncover and stand for 5 mins before serving.
Creamy Chicken Bake
Serves 8

Ingredients:
- 1 tbs olive oil
- 20g butter
- 1.2kg chicken thigh fillets, cut into 4cm pieces
- 2 leeks, trimmed, sliced
- 200g middle bacon rashers, chopped
- 250g cup mushrooms, thickly sliced
- 1/2 cup dry white wine
- 1/3 cup plain flour
- 3/4 cup chicken stock
- 3/4 cup pure cream
- 80g baby spinach
- 1.6kg potatoes, peeled, chopped
- 100g butter, chopped
- 1/2 cup full cream milk

Directions:
1. Make mashed-potato topping; place potato in a large saucepan. Cover with cold water. Bring to the boil. Simmer over medium heat for 10 to 15 minutes or until tender. Drain. Return to pan. Add butter and milk. Mash until smooth.
2. Preheat oven to 200°C/180°C fan-forced. Heat oil and butter in a large pan over a medium-high heat. Cook chicken, in batches, for 2 to 3 minutes or until browned. Transfer to a 6cm-deep, 3.8 litre-capacity baking dish.
4. Season with salt and pepper. Place spinach over chicken in dish. Carefully pour cream mixture over spinach. Stir to combine. Top evenly with mashed potato. Bake for 25 to 30 minutes or until golden.
Cheesy Cauliflower Puree
Yield: 2 cups

Ingredients:
1 head of cauliflower
3 tbs heavy cream
2 tbs butter
75 grams of a sharp cheese
salt and pepper to taste

Directions:
1. Clean and trim the cauliflower, breaking it into medium sized pieces.
2. Place in a microwave safe bowl with cream and butter.
3. Microwave, uncovered, on high for six minutes.
4. Stir to coat cauliflower with cream/butter mixture.
5. Microwave for another six minutes on high.
6. Remove from the microwave and put into a high speed blender or food processor along with the cheese. Puree until smooth.
7. Season with salt and pepper to taste.
Avocado & Berry Power Smoothie
Yield: 2 cups

Ingredients:
1/2 firm ripe avocado, peeled
125g (1 cup) frozen mixed berries
325ml (1 1/2 cups) full fat milk (cow, soy, or coconut)
2 teaspoons honey
1 tablespoon LSA meal

Directions:
1. Place all ingredients in a blender and blend until smooth and creamy.
2. Divide between 2 glasses, serve immediately.

Tip: LSA meal is a blend of ground linseeds, sunflower seeds and almonds, it is a great source of healthy fats. You can find it in the health food section of your supermarket.
Coconut Banana Pancakes & Lemony Butter
Makes 4 Large Pancakes or 10 Small Pancakes

Ingredients:
1 medium banana, roughly chopped
2 eggs
6-7 tbs almond meal
3 tbs desiccated coconut, 1/2 tsp baking powder
1/2 tbs vanilla essence
1/2 flour
1/2 milk
oil or butter for cooking

Lemon butter Ingredients:
2 tbs butter
1/2 lemon, zest and juice
handful of blueberries
maple syrup to serve

Directions:
Grate butter into a bowel and combine with grated lemon zest and lemon juice. Use a fork to mash the butter and incorporate lemon. Set aside.

Place all ingredients in a blender and puree until smooth and fluffy. If you don’t have a blender, you can mash banana with a fork and whisk the eggs in a bowl and then combine with the rest of ingredients.

Heat up a generous dollop of oil or butter in a frying pan. Cook pancakes in batches.
You know it’s time to turn them over when little bubbles start appearing in the batter.
Add a little more oil/butter in between pancakes.

Serve with lemon butter or/and maple syrup and berries.
Weight Maintenance on a Low Fat Diet

Deakin University Research Study

The low fat diet is designed to reduce your fat intake, but is not a weight loss diet. Therefore, we ask that you increase the amount of carbohydrate and protein you are eating to replace the energy you would normally receive from fat. You should also weigh yourself once or twice a week to check if your weight is stable.

Ways to increase your calories/energy intake on a low fat diet:

- Eat regular meals (breakfast, lunch, dinner) plus mid-meal snacks: 3 meals and 3 snacks a day.
- Sip on high carbohydrate/energy drinks e.g. soft drink, cordial, skim milk, homemade smoothies and juice.
- Snack on dried fruit and add it to cereals and yogurt.
- Add skim milk powder to cereals, sauces, desserts, mashed vegetables, soup and drinks.
- Add sugar, honey or syrup to cereals, yoghurts, breads, fruit and drinks.
- Eat lollies such as Minties, musk-sticks, lollipops, marshmallows, jelly beans, gummie fruits, Turkish delight, liquorice, and/or spearmint leaves at the end of a meal or as a snack.
- Increase the portion size of carbohydrate rich foods (i.e. rice, pasta, legumes, potato, bread) in your meal, in place of fat-containing foods.
- Include high carbohydrate and easy-to-digest foods such as potato, toast, rice, pasta, low-fat crackers, pretzels or unbuttered popcorn.
Low fat, high energy foods:

- Jelly
- Sorbet
- Boiled lollies
- Jelly lollies
- Jam on English muffins
- Honey on crumpets
- Marmalade on toast
- Vegemite and cottage cheese on toast
- Low-fat bagels with honey and low-fat ricotta cheese
- Unbuttered popcorn
- Low-fat pretzels
- Skim milk
- Fat-free yoghurt
- Smoothies with fruit and fat-free yoghurt
- Plain breads e.g. white, wholemeal, rye, pita bread
- Breakfast cereals, especially sweetened cereals e.g. Nutrigrain, Coco Pops, Honey Weets, Fruit Loops
- Pasta with a tomato based sauce and cottage cheese/ beans or tofu
- Low-fat crackers; Ryvita, light Premiums/Salads, Cruskits, rice cakes, corn thins, rice crackers
- Fruit juice
- Vegetable juice
- Soft drink
- Cordial
- Sports drinks
- Skim milkshake (no ice cream)
- Dried fruit
- Canned fruit in juice or syrup
- Fruit salad with fat-free yoghurt and sugar
- Pancakes with maple syrup or lemon and sugar
- Low-fat puddings and mousse e.g. Hunts Fat-Free Chocolate Pudding, Weight Watchers Instant mousse or Nestle Soleil Diet Desserts.
- Baked beans on toast
- Tinned spaghetti jaffle
- Veggie burgers
- Lentils and beans e.g. Dahl
- Low fat rice and couscous dishes e.g. pilaf
Zucchini Egg White Omelette

Serves 1

Ingredients:

5 egg whites, lightly beaten
1 small zucchini, grated
50g low-fat cottage cheese
1 small handful fresh thyme, leaves picked, to garnish (optional)

Directions:

1. Put the beaten egg whites and grated zucchini in a jug. Season with salt and pepper.

2. Pour the mixture in a small non-stick frying pan over a medium-high heat. Swirl the mixture around the pan to cover the base. Gently slide a knife under the edges of the omelette.

3. When the omelette is beginning to cook around the edges, scatter over the cottage cheese, so that it is covered evenly. Continue cooking until the centre is almost cooked, but still just a little wet. Remove from the heat, and leave for a couple of minutes to set – the retained heat will continue to cook the omelette.

4. Sprinkle over a little black pepper, and garnish with thyme leaves (if using). Carefully slide out of the pan, and serve immediately.
Pasta with Cannellini Beans and Cottage Cheese
Serves 3

Ingredients:

- 400g pasta, any shape
- 1 small onion, finely chopped
- 2 garlic cloves, minced
- 400g can chopped tomatoes, with juice
- 1 cup low-fat cottage cheese
- 1 tbsp of sugar
- 400g can cannellini beans, drained and rinsed
- 4 sage leaves or basil leaves, cut in slivers
- Salt and ground pepper

Directions:

1. Bring a large pot of water to a boil.
2. Meanwhile, cook the onion in a non-stick skillet or saucepan. Add the garlic, and stir together until fragrant, about 30 seconds. Add the tomatoes with juice and sugar. Raise the heat slightly, and cook, stirring, until the tomatoes are bubbling vigorously.
3. Lower the heat to medium-low, and cook gently, stirring and mashing the tomatoes often with the back of your spoon until they have cooked down into a thick, fragrant sauce, 15 to 20 minutes. Stir in the beans and the herbs, and season to taste with salt and pepper. Keep warm.
4. When the pasta water comes to a boil, add the pasta. Cook al dente, following the recommendations on the package.
5. To serve, add a serving of pasta to a plate. Top with 1/3 cup cottage cheese and pasta sauce. Serve immediately.
Sweet Tofu and Pineapple Fried Rice

Serves 2

Ingredients:

- 1 tsp olive oil
- 1/2 cup diced onion
- 1/2 cup diced red capsicum
- 1 tbsp soy sauce
- 1 tbsp sweet chilli sauce
- 1 tbsp brown sugar
- 300g firm tofu (drained and cut into 2cm cubes)
- 250g can pineapple chunks in juice, drained
- 2 cups cooked rice
- 2 spring onions chopped
- 4 lime wedges

Directions:

1. Heat the oil in a non-stick wok over a medium heat. Add the onion and capsicum and sauté for 3-5 minutes or until the onion softens.
2. Add sauces, sugar, tofu, pineapple, rice and spring onions, stir, cook for about 5 minutes more, until combined.
3. Serve with lime wedges
BBQ Tofu Sandwich
Serves 4

Ingredients:

1/4 cup thinly sliced onion
500g extra-firm or firm water-packed tofu, drained
1/4 teaspoon salt
3/4 cup barbecue sauce
1 1/2 cups coleslaw mix or finely shredded cabbage
2 teaspoons red wine vinegar
2 tablespoons fat-free mayonnaise e.g. Kraft Fat-Free or Praise 99% Fat-Free
1/4 teaspoon garlic powder
Freshly ground pepper to taste
4 hamburger buns, toasted
2 tablespoons sweet chilli sauce
4 dill pickle slices

Directions:

1. Place onion in a small bowl, cover with cold water and set aside. Stand tofu on its long narrow side. Cut lengthwise into 4 rectangular slabs, each about 2cm thick, and pat dry. Sprinkle with salt.

2. Heat the tofu slabs in a large non-stick skillet over medium heat. Cook until browned on both sides, about 4 minutes per side. Reduce heat to low. Add barbecue sauce and carefully turn the tofu to coat with the sauce. Cover and cook for 3 minutes more.

3. Combine coleslaw (or cabbage), mayonnaise, vinegar, garlic powder and pepper in a medium bowl. Drain the onion.

4. To assemble sandwiches, spread sweet chilli sauce on the bottom of each bun, place about 1/3 cup of the coleslaw mixture and top with a tofu slab, a pickle slice and a few onion slices. Spread any sauce remaining in the pan on the top buns.
Banana-Mango Smoothie
Yield: 2 cups

Ingredients:
1 peeled and ripe mango, cubed
1 large ripe banana, sliced
1/2 cup fat-free natural/vanilla yoghurt
2/3 cup skim milk
1 tablespoon skim milk powder (optional)
2 teaspoon honey or sugar

Directions:
1. Blend mango, banana, yoghurt, skim milk, skim milk powder and honey/sugar in a blender until smooth.

Tropical Smoothie
Yield: 2 cups

Ingredients:
1 cup fresh or canned pineapple
1 large ripe banana, sliced
2 tablespoons canned passion fruit pulp
1 peeled and ripe mango, cubed
1 cup lime/coconut sorbet
2/3 cup tropical/orange juice

Directions:
1. Blend pineapple, banana, passionfruit, mango, sorbet and juice in a blender until smooth
Low-fat Cracker Toppings

1. Prawns with mango salsa (mango, red chilli, lime juice, soy sauce, sugar and coriander)

2. Low-fat ricotta dusted with cinnamon, topped with banana and honey

3. Tuna, tomato, cucumber, lime juice, sugar and Spanish onion.

4. Sliced strawberries, low-fat ricotta cheese, balsamic vinegar, sugar and mint

5. Low-fat hummus dip with roasted capsicum and roasted onion.

6. Egg whites with fat-free mayo and seeded mustard
Crushed Pea Cruskits

Serves 1

Ingredients:

- 100g frozen peas
- 2g garlic, finely chopped
- Squeeze lemon juice
- 1 tablespoon sugar
- 40g spring onions, sliced
- 5g mint leaves, finely chopped
- Salt and pepper to taste
- 4 Cruskits

Directions:

1. Soak the peas in boiling water for one minutes to defrost.

2. In a mini blender combine the peas, garlic, sugar and lemon juice, and pulse until combined. Stir through the chopped spring onions and mint. Season to taste and serve on the Cruskits.

Other Low-Fat Cracker Toppings

Chutney, pickle, relishes, mustard, tomato, beetroot, low-fat ricotta cheese, low-fat cottage cheese, salsa, low-fat mayonnaise, low-fat dips e.g. hummus, vegemite, jam, marmalade, honey, baked beans, canned tuna or salmon in brine.
Low-fat Hummus Dip

Makes 1 cup

Ingredients:

- 400g can chickpeas, rinsed, drained
- 60ml lemon juice
- 1 teaspoon tahini
- 2 tablespoons water
- 1 teaspoon ground cumin
- 1/2 teaspoon ground coriander
- 1 small garlic clove, crushed
- Salt & black pepper
- Sweet paprika, to garnish

Directions:

1. Place chickpeas, lemon juice, tahini, water, cumin, coriander and garlic in the bowl of a food processor and process until a smooth paste forms. Taste and season with salt and pepper. (Add a little extra lemon juice or water if the hummus is too thick.)

2. Transfer hummus to a bowl. Sprinkle with paprika to garnish. Serve with low-fat crackers, pita bread, pita chips or in a sandwich.

Pita Chips

Directions:

1. Preheat oven to 200 degrees C.

2. Slice pita bread into triangles. Place triangles on a baking tray lined with baking paper.

3. Bake in the preheated oven for about 8 minutes, or until lightly browned and crispy.
Appendix Q – High-Fat Weight Maintenance Booklet

Weight Maintenance on a High Fat Diet

Deakin University Research Study

The high fat diet is designed to increase your fat intake, but it is not intended that you gain weight while on this diet. Because you are eating foods with more fat it is likely that you will need to reduce the amount of starchy and sugary foods you consume. You should also weigh yourself once or twice a week to check if your weight is stable.

Weigh maintenance on a moderate fat diet:

- Eat three high-fat meals a day and limit mid-meal snacks.
- Reduce your intake of sugary drinks such as soft drink, cordial, sports drinks and juice. Instead choose: water, tea, coffee, diet soft drink, unflavoured mineral water and/or diet cordial.
- Eat larger servings of fried meat, chicken and fish and smaller portions of starchy foods e.g. rice, pasta, legumes, potato and bread.
- Limit your intake of high carbohydrate snacks such as dried fruit, toast, cereal, rice cakes, lollies and sorbet.
- If you like desserts you can include high-fat desserts (e.g. cheesecake, custard or chocolate) but have a smaller serving of the main course.

It may help to base your diet on the following foods:

- **Meat:** Beef, lamb, pork, chicken, sausages, etc.
- **Fish:** Salmon, trout, tuna, sardines, mackerel, etc.
- **Eggs:** Omelettes, scrambled, fried, etc.
- **Nuts, Seeds & Oils:** Almonds, walnuts, sunflower seeds, olive oil, etc.
- **Full-Fat Dairy:** Milk, cheese, heavy cream, sour cream, yogurt, etc.
- **Vegetables:** Avocado, spinach, broccoli, cauliflower, tomato, carrots, etc.
Snack options:

- 1/3 cup or 30g nuts (equivalent to; 20 almonds, 15 cashews, 15 pecans, 15 macadamias, 10 walnuts or 2 tbsp. pine nuts)
- 1/3 cup or 30g seeds e.g. sunflower, sesame or pumpkin
- 1/2 avocado
- 125 - 175g full-fat yoghurt e.g. Gippsland Yoghurt Twist, Allure, Danone Greek Style, Dairy Farmers Thick & Creamy, Ski Divine, Jalna Premium, Five:am Organic Yoghurt and Tamar Valley Greek Yoghurt *where possible choose ‘No Added Sugar’ varieties
- 300 - 600ml full cream milk (plain or flavoured) e.g. Farmers Union One, Dare, OAK and Dairy Farmers Classic
- Avocado smoothie (recipe in ‘Following a High Fat Diet’ booklet)
- 20 - 30g or 7-10 medium olives
- 40g or 2 slices of cheese
- 2 - 3 slices of salami on a buttered Salada
- Peanut butter or avocado on a Vita-Weat lunch slice
- Peanut butter or cream cheese on celery
- 100g coleslaw or potato salad made with oil/egg based mayonnaise
- 25g or 6 squares of chocolate
- 2 hard-boiled eggs/devilled eggs
- 1 sweet biscuit e.g. Scotch Finger, Tim Tam, Butternut Cookie or Shortbread Cream
- 4 - 6 savoury biscuits e.g. Cheds, Country Cheese, Ritz, Jatz, Clix
- Full-fat dip with 4-5 savoury biscuits or veggies sticks
- Guacamole and 8 - 10 fried corn chips
- Smoked salmon with cream cheese
- 90g can of tuna or salmon in oil
- Muesli bar e.g. Uncle Tobys (Yoghurt Topps/ Oat Slice/ Chewy), Carmans Nut Bars, Nice & Natural Nut Bars, Mother Earth Golden Baked, Be Natural Dark Chocolate/Nut Bars, Nature Valley Crunchy Bars and Thankyou Nut Bars.
A sample menu:

**Monday**

**Breakfast:** 2 - 3 egg omelette with cheese and spinach, fried in butter or oil.

**Lunch:** 1 pasty, pie or sausage roll

**Dinner:** Cheeseburger with egg (no bun), served with zucchini gratin (see recipes) or buttered carrots and beans.

  + 1 - 2 snack options from previous page (optional)

**Tuesday**

**Breakfast:** Croissant with ham & cheese

**Lunch:** 200g full-fat Greek yogurt with toasted muesli, coconut flakes and a serve of nuts

**Dinner:** Salmon or crumbed fish with cauliflower cheese or broccoli casserole (see recipes)

  + 1 - 2 snack options from previous page (optional)

**Wednesday**

**Breakfast:** Smashed avocado (1/2 an avocado and 40g fetta cheese)

**Lunch:** 2 fried fish cakes with buttered cabbage (see recipes)

**Dinner:** Fried chicken/veal schnitzel with fried potato wedges and avocado salsa (see recipes)

  + 1 - 2 snack options from previous page (optional)

**Thursday**

**Breakfast:** 2 rashers of bacon, 2 eggs and 1 fried tomato (optional)

**Lunch:** Smoked salmon and avocado salad with oil-based dressing

**Dinner:** Porterhouse steak and cheesy cauliflower mash (See recipes)

  + 1 - 2 snack options from previous page (optional)
Appendices

**Friday**

**Breakfast:** Full-fat yoghurt/milk with toasted muesli

**Lunch:** Quiche Lorraine (recipe in 'Following a High Fat Diet' booklet)

**Dinner:** Fried pork chops & mashed potato with added butter/cream

+ 1 - 2 snack options from previous page (optional)

**Saturday**

**Breakfast:** Coconut pancakes and lemon butter (recipe in 'Following a High Fat Diet' booklet)

**Lunch:** Nachos with cheese, guacamole & sour cream

**Dinner:** Meatballs in mushroom sauce with creamy polenta (see recipes)

+ 1 - 2 snack options from previous page (optional)

**Sunday**

**Breakfast:** Egg Benedict muffin (1 egg, 1 slice ham/bacon, 1 tbsp. hollandaise sauce, 1 buttered English muffin)

**Lunch:** Avocado baked egg (see recipes)

**Dinner:** Chicken wings and fried cabbage with onion and bacon (see recipes)

+ 1 - 2 snack options from previous page (optional)
Side Dish: Zucchini Gratin
Serves 4

Ingredients:
- 500g zucchini, shredded on the large holes of a grater
- 1 tsp. salt
- 2 tbsp. butter
- 1/2 small onion, finely diced
- 1/2 cup double cream
- 1/2 cup shredded Gruyere cheese

Directions:
1. Put the shredded zucchini in a colander, and toss with 1/2 tsp. salt. Let stand for at least 5 minutes, then squeeze as much of the liquid out of the zucchini as possible using your hands.

2. Melt the butter in a saute pan. Add the onions, and cook until softened. Add the zucchini, stir, and cook for a few more minutes until the zucchini is just softened. Add the cream, and simmer until thickened.

3. Remove the mixture from the heat, and season to taste with salt and pepper. Transfer the mixture to a baking dish, and top with the shredded Gruyere.

4. Bake in a 350 degree oven until the cheese is melted and the everything is heated through.
Side Dish: Broccoli Casserole
Serves 6

Ingredients:
3 tbsp. butter 1 egg, beaten
1/2 onion, chopped 1/4 tsp. garlic
400g chopped broccoli 1/8 tsp. black pepper
300g can condensed cream of mushroom soup 1/4 tsp. salt
1 cup shredded Cheddar cheese 1 tsp. lemon juice
1/2 cup whole egg mayonnaise 10 buttery crackers, crushed fine

Directions:
1. Preheat oven to 175 degrees C
3. In a casserole dish, mix together onion, broccoli, soup, cheese, mayonnaise, eggs, garlic, pepper, salt and lemon juice. Sprinkle crushed crackers over top and dot with remaining 1 tbsp. butter.
4. Bake uncovered in preheated oven for 45 minutes, until heated through and browned on top.
Fried Fish Cakes
Serves 4

Ingredients:
- 500g mackerel fillets, cut into cubes
- 1 tbsp. fish sauce
- ½ tsp. salt
- ½ tsp. freshly ground pepper
- 1 tsp. sesame oil
- 1 egg, beaten
- 1 garlic clove, finely diced
- 5 spring onions, white part only, finely sliced
- 2 tbsp. flour
- A generous knob of butter
- Vegetable oil, for deep frying
- Nuoc mam cham (dipping fish sauce) or lemon wedges, to serve

Directions:
1. Cut the mackerel into small pieces, then grind it up in a food processor or pound it in a mortar and pestle until it's a paste.

2. Transfer to a large mixing bowl, and add fish sauce, salt, pepper, sesame oil, egg, garlic and spring onion.

3. Using oiled hands, knead the mixture until sticky enough to form it into patties about 8 cm wide and 2cm thick. Coat in flour.

4. Heat a frying pan with the butter and oil until the butter begins to foam. Add the fish patties in batches and fry for 4 minutes on each side until golden.

5. Serve with nuoc mam cham for dipping or lemon wedges.
Side Dish: Buttered Cabbage
Serves 4

Ingredients:
1 tsp. olive oil
1 onions, sliced thinly
1 medium cabbage (-800g), shredded
1/4 cup water
3-4 tbsp. butter
salt and pepper to taste

Directions:
1. Heat oil in a large skillet over medium heat. Add the sliced onion and sauté for a couple of minutes.
2. Add the shredded cabbage to the pan with a generous pinch of sea salt. Sauté for about five minutes, stirring occasionally.
3. Add the water to the skillet, cover with a lid and allow to steam for 5-10 minutes, or until cabbage has softened. Remove lid, simmer until most of the moisture has evaporated, and remove from heat.
4. Add butter and sea salt and pepper to taste. Serve as a side dish.
Side Dish: Fried Potato Wedges
Serves 4

Ingredients:
1 cup full cream milk 1/4 tsp. salt
1 egg, beaten 2 tbsp. grated parmesan cheese
1/2 cup flour 3 medium potatoes, cut into wedges
1 tsp. garlic salt 5 tbsp. vegetable oil (for frying)
3/4 tsp. pepper Salt and pepper
1/2 tsp. dried mixed herbs

Directions:
1. Wisk the egg and milk together in a large bowl until well blended.
2. Mix the dry ingredients and parmesan together in a separate large bowl.
3. Dip potato wedges into the egg and milk, then into the flour mixture. Toss meticulously to coat the potatoes evenly.
4. Heat the oil in a large skillet over medium heat. Add the potato wedges once small bubbles appear in the oil.
5. Cover the pan and cook the potato wedges for 10 minutes. Stir the wedges every three minutes so they don't stick to each other or the pan.
6. Remove the wedges from the pan and lay them on a layer of paper towels.
7. Sprinkle the wedges with salt and pepper to taste. Serve warm.

* Can also be eaten as a snack with sour cream or guacamole
Side Dish: Avocado Salsa
Serves 4

Ingredients:
2 medium avocados, diced 1/2 red onion, finely diced
1 lime, juiced 1/3 cup coriander leaves, roughly chopped
1 tablespoon olive oil Salt and pepper
10 - 12 cherry tomatoes, chopped

Directions:
1. Place avocado in a bowl. Spoon over lime juice and oil. Toss gently to coat.
2. Stir together with remaining ingredients.
3. Season to taste with salt and pepper
Side Dish: Cheesy Cauliflower Mash
Serves 4

Ingredients:
1 small head of cauliflower (≈250g), chopped into small pieces
4 tbsp. full fat cream cheese
1/4 cup full cream milk
1/2 cup shredded cheddar cheese
2 tbsp. parmesan cheese
1/4 tsp. pepper
2 tsp. butter
1/4 tsp. salt
1 tsp. paprika
1 tsp. minced garlic
1 tbsp. fresh rosemary, finely chopped

Directions:
1. Steam the cauliflower for 10 to 12 minutes, until just tender. Place the cauliflower in a large bowl and mash with a potato masher or fork.
2. In another large bowl combine the remaining ingredients.
3. Pour the hot mashed cauliflower on top of the cheese mixture and combine, using a large spoon, until the cheeses are thoroughly melted. Serve immediately.
Meatballs in Mushroom Sauce
Serves 5

Ingredients for the meatballs:
2 thick slices of wholemeal bread
100ml full-cream milk
250g regular beef mince
200g pork shoulder mince
1 egg, beaten
1 small shallot, peeled and chopped
1 small clove garlic, crushed
Salt and pepper
2 tbsp. olive oil
1 tbsp. butter

Ingredients for the Mushroom sauce:
300ml chicken or vegetable stock
100ml dry white wine
1 small shallot, peeled and chopped
1 clove garlic, crushed
1 fresh bay leaf
100g button mushrooms, finely sliced
250ml double cream
Lemon juice, to taste
Salt and pepper
Grated parmesan cheese

Directions:
1. For the pork and beef meatballs, remove the crusts from the bread. Place into a bowl and pour over the milk. Leave to stand for five minutes.
2. Place the beef and pork mince into a blender along with the shallot, garlic, and seasoning.
3. Squeeze the excess milk from the bread, discarding the milk, and add bread to the blender. Blend until it forms a thick mixture.
4. With slightly wet hands form the meat mixture into balls the size of a small walnut.
5. Heat a non-stick pan and add olive oil and butter.
6. Fry the balls in batches rolling them in the pan until they are evenly browned; this will take between 6-8 mins per batch.
7. Lift the balls out of pan and place in a non-stick roasting tray.
8. Heat the oven to 180C. Put the meatballs into the oven to finish cooking for 15 minutes.
9. For the mushroom sauce, place the stock, wine, shallot, garlic, bay leaf and mushrooms into a saucepan and bring to the boil. Reduce the heat and simmer for 40 minutes.

10. Pour in the cream and bring it back to a simmer and continue cooking for a few minutes until it is the consistency of a thin sauce. Adjust the seasoning with the lemon juice, salt and freshly ground black pepper.

11. Remove meatballs from oven. Dish up alongside the creamy parmesan polenta. Spoon over the mushroom sauce and serve immediately.

Side Dish: Creamy Parmesan Polenta
Serves 4

Ingredients:
250ml (1 cup) cooking cream
750ml (3 cups) cold water
170g (1 cup) instant polenta
40g (1/2 cup) finely grated parmesan
40g butter
Salt and pepper

Directions:

2. Using a balloon whisk, whisk polenta vigorously for 2 minutes or until thickened. Remove from heat and stir with a wooden spoon for 1 minute.

3. Add butter, three-quarters of the grated parmesan and salt and pepper. Stir until butter melts. Top with remaining parmesan and serve.
**Avocado Baked Egg**
Serves 2

**Ingredients:**
- 1 avocado, halved and pitted
- 2 eggs
- Salt and pepper to taste
- 1 pinch cayenne pepper
- 1/4 cup crumbled cooked bacon or parmesan cheese

**Directions:**
1. Preheat the oven to 220 degrees.
2. Place each avocado half in a ramekin. Crack 1 egg into each avocado half; season with salt, black pepper, and cayenne pepper. Place ramekins on a baking sheet.
3. Bake in the preheated oven until entire egg is cooked through, about 15 minutes. Sprinkle each avocado with bacon and/or parmesan cheese.
**Side Dish: Fried Cabbage with Bacon**

**Serves 4**

**Ingredients:**

<table>
<thead>
<tr>
<th>1 onion, sliced</th>
<th>1 clove garlic, minced</th>
</tr>
</thead>
<tbody>
<tr>
<td>50g butter</td>
<td>2 tbsp. olive oil</td>
</tr>
<tr>
<td>6 rashers of bacon, chopped</td>
<td>1 tsp. salt, or to taste</td>
</tr>
<tr>
<td>1 medium head cabbage</td>
<td>1 tsp. pepper</td>
</tr>
<tr>
<td>(-800g), cored and sliced</td>
<td>1/4 tsp. paprika</td>
</tr>
</tbody>
</table>

**Directions:**

1. Fry the onion in butter for 2 mins, then add the bacon and cook for a few mins more until golden.

2. Stir in the cabbage and garlic. Cook for 2 mins over a medium heat until wilted, then cover, turn the heat to low and cook for another 5 mins.

3. Season with salt, pepper and paprika.

4. Uncover and stir well; the cabbage should have a little colour. If not, increase the heat and cook for a few moments more.
### Nutrition Information Tables:

#### Avocado Baked Egg

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<thead>
<tr>
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<th>Per 100g</th>
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<td>Energy (kJ)</td>
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#### Avocado Salsa

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#### Broccoli Casserole

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### Buttered Cabbage

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### Cheesy Cauliflower Mash

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### Creamy Parmesan Polenta

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### Fried Cabbage with Bacon

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### Fried Fish Cakes

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<td>Carbohydrate (g)</td>
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### Fried Potato Wedges

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<tr>
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<td>Protein (g)</td>
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<td>Total fat (g)</td>
<td>27.72</td>
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<td>Carbohydrate (g)</td>
<td>28.12</td>
<td>13.24</td>
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### Meatballs in Mushroom Sauce

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<tr>
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</thead>
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<tr>
<td>Energy (kJ)</td>
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<tr>
<td>Protein (g)</td>
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<td>8.99</td>
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<td>Total fat (g)</td>
<td>52.29</td>
<td>17.32</td>
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<tr>
<td>Carbohydrate (g)</td>
<td>3.95</td>
<td>1.31</td>
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### Zucchini Gratin

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<tr>
<td>Energy (kJ)</td>
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<tr>
<td>Protein (g)</td>
<td>4.58</td>
<td>2.38</td>
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<td>Total fat (g)</td>
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<td>15.82</td>
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<tr>
<td>Carbohydrate (g)</td>
<td>2.72</td>
<td>1.59</td>
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Food Diary

Instructions:
- Be as specific as possible with brand names and amounts
- Please write every different food / drink on a new line and leave a line in between each entry e.g.

<table>
<thead>
<tr>
<th>Meal</th>
<th>Time</th>
<th>Food/drink</th>
<th>Quantity</th>
<th>Cooking method eg Fried, steamed, raw, not applicable (na) etc</th>
<th>Additions (ie salt added, butter added, salad dressing added etc)</th>
</tr>
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<tbody>
<tr>
<td>B/F</td>
<td>7am</td>
<td>Kelloggs Cornflakes</td>
<td>1 cup</td>
<td>na</td>
<td>none</td>
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<td></td>
<td></td>
<td>Milk – Pura light start</td>
<td>½ cup</td>
<td>na</td>
<td>none</td>
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<tr>
<td></td>
<td></td>
<td>Sugar – white, table sugar</td>
<td>2 tsp</td>
<td>na</td>
<td>none</td>
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<tr>
<td></td>
<td></td>
<td>Tea – tea bag, weak</td>
<td>200 ml</td>
<td>na</td>
<td>none</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Milk – Skinny milk</td>
<td>2 tblsp</td>
<td>na</td>
<td>none</td>
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<tr>
<td></td>
<td></td>
<td>Sugar – brown</td>
<td>1 tsp</td>
<td>na</td>
<td>none</td>
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<tr>
<td></td>
<td></td>
<td>White Bread thick slice</td>
<td>2 slice</td>
<td>toasted</td>
<td>butter</td>
</tr>
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<td></td>
<td></td>
<td>Eggs – Coles cage</td>
<td>2</td>
<td>poached</td>
<td>Salt, pepper, tomato sauce</td>
</tr>
<tr>
<td>Meal</td>
<td>Time</td>
<td>Food/drink</td>
<td>Quantity</td>
<td>Cooking method eg Fried, steamed, raw, not applicable (na) etc</td>
<td>Additions (ie salt added, butter added, salad dressing added etc)</td>
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</tbody>
</table>
Appendix S – Food Frequency Questionnaire

School of Health Sciences

DEAKIN

Appendices

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.

<table>
<thead>
<tr>
<th>Example</th>
<th>Average number of times consumed in the last month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pineapple</td>
<td>1-4 times per month</td>
</tr>
</tbody>
</table>

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 for 4-5 times per day for wholemeal/mixed grain bread etc and the ‘Once per day’ box for white bread etc.

If you usually eat a banana at breakfast seven times a week and an apple at lunch three times a week, and you usually eat no other bananas or apples during the week, fill in the box for ‘Once per day’ for bananas and the ‘4-5 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.
### Appendices

#### Section one

For each food listed, fill in a box indicating how often on average you consumed that food in the past month.

<table>
<thead>
<tr>
<th>Food Description</th>
<th>Never, or less than once a month</th>
<th>1-3 times per month</th>
<th>Once per week</th>
<th>2-4 times per week</th>
<th>5-6 times per week</th>
<th>Once per day</th>
<th>2-3 times per day</th>
<th>4-5 times per day</th>
<th>6+ times per day</th>
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<tbody>
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<tr>
<td>Flavoured milk drink (e.g. milkshake, iced coffee, hot chocolate)</td>
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<td>Milk as a drink</td>
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<td>Milk in hot beverages (e.g. in coffee, tea)</td>
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<td>Cream or sour cream</td>
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<td>Yoghurt, plain or flavoured (including fromage frais)</td>
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<td>Cottage or ricotta cheese</td>
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<td>Cheddar and other cheeses</td>
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<tr>
<td>White bread, toast or rolls</td>
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<td>Wholemeal/mixed grain bread, toast or rolls</td>
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<td>English muffins, bagel or crumpet</td>
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<td>Dry or savoury biscuits, crispbread, crackers</td>
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<td>Rice (including white or brown)</td>
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<td>Pasta (including filled), noodles</td>
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<td>Meat, Fish, Eggs</td>
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<td>Mince dishes (e.g. rissoles, meat loaf)</td>
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<td>Mixed dishes with beef, veal (e.g. casserole, stir fry)</td>
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<td>Beef, veal - roast, chop or steak</td>
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<td>Mixed dishes with lamb (e.g. casserole, stir fry)</td>
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<td>Lamb - rustic, chop or steak</td>
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<td>Mixed dishes with pork (e.g. casserole, stir fry)</td>
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<td>Pork - roast, chop or steak</td>
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<td>Sausage, frankfurter</td>
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<td>Bacon</td>
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<td>Ham</td>
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<td>Luncheon meats, salami</td>
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<td>Liver (including pate)</td>
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8005 640283
### Appendices

#### For each food listed, fill in the box indicating how often, on average, you consumed that food in the last month.

<table>
<thead>
<tr>
<th>Food Description</th>
<th>Never, or less than once a month</th>
<th>1-3 times per month</th>
<th>Once per week</th>
<th>2-4 times per week</th>
<th>5-6 times per week</th>
<th>Once per day</th>
<th>3-5 times per day</th>
<th>4-5 times per day</th>
<th>6+ times per day</th>
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<tbody>
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<td>Other offals (e.g. kidneys)</td>
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<tr>
<td>Mixed dishes with chicken, turkey, duck (e.g. casserole, stir-fry)</td>
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<td>Chicken, turkey, duck - roast, steamed, BBQ</td>
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<td>Canned tuna, salmon, sardines</td>
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<td>Fish, steamed, baked, grilled</td>
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<td>Other seafood (e.g. prawns)</td>
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<td><strong>Sweets, Baked Goods and Snacks</strong></td>
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<td>Cakes, sweet muffins, scones or pikelets</td>
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<td>Other puddings or desserts</td>
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<td>Cream, chocolate biscuits</td>
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<td>Meat pie, sausage roll or other savoury pastries</td>
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<td>Chocolate (including chocolate bars, e.g. Mars bars)</td>
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<td>Jam, marmalade, syrup or honey</td>
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<td>Vegemite, Marmite, Promite</td>
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<td>Potato chips, corn chips, Twixels, etc</td>
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<td>Mayonnaise or other creamy dressing</td>
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<td><strong>Non-dairy Beverages</strong></td>
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<td>Vegetable, tomato juice</td>
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<td>Fruit juice drink or fruit drink</td>
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<tr>
<th>Food Description</th>
<th>Never, or less than once a month</th>
<th>1-3 times per month</th>
<th>Once per week</th>
<th>2-4 times per week</th>
<th>5-6 times per week</th>
<th>Once per day</th>
<th>2-3 times per day</th>
<th>4-5 times per day</th>
<th>6+ times per day</th>
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<tr>
<td>Low/lowe soft drink</td>
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<td>Soft drinks (including flavoured mineral water)</td>
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<td>Water (including unflavoured mineral water, soda water, tap water)</td>
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<td>Soy beverages</td>
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<td>Beer - low alcohol</td>
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<td>Beer - ordinary</td>
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<td>Red wine</td>
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<td>White wine or champagne/sparkling wine</td>
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<td>Sherry or port</td>
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<td>Spirits, liqueurs</td>
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<td>Vegetables (including frozen and fresh)</td>
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<td>Green mixed salad (including lettuce, tomato, etc)</td>
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<td>In a sandwich</td>
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<tr>
<td>As a side salad with a main meal</td>
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<td>Stir fried or mixed vegetables</td>
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<td>Vegetable casserole</td>
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<tr>
<td><strong>Excluding their use in the above mixed dishes, please indicate how often you eat the following vegetables</strong></td>
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<tr>
<td>Potato, boiled, mashed, baked</td>
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<td>Hot chips</td>
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<td>Pumpkin</td>
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<td>Sweet potato</td>
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<td>Peas</td>
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<td>Green beans</td>
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<td>Silverbeet, spinach</td>
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<td>Broccoli</td>
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<td>Cauliflower</td>
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<tr>
<td>Brussel sprouts, cabbage, coleslaw</td>
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<td>Carrots</td>
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### Average number of times consumed in the last month

<table>
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<th>Food or Supplement</th>
<th>Never, or less than once a month</th>
<th>1-3 times per month</th>
<th>Once per week</th>
<th>2-4 times per week</th>
<th>5-8 times per week</th>
<th>Once per day</th>
<th>2-3 times per day</th>
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<tr>
<td>Zucchini, eggplant, squash</td>
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<td>Sweetcorn, corn on the cob</td>
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<td>Tomatoes</td>
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<td>Lettuce</td>
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<tr>
<td>Celeriac, cucumber</td>
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<td>Onion or leek</td>
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<tr>
<td>Soybeans, tofu</td>
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<td>Baked beans</td>
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<tr>
<td>Other beans, lentils</td>
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<tr>
<td>Fruits (including dried, frozen and tinned)</td>
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<tr>
<td>Apple or pear</td>
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<tr>
<td>Orange, mandarin or grapefruit</td>
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<tr>
<td>Banana</td>
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<tr>
<td>Peach, nectarine, plum or apricot</td>
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<tr>
<td>Mango or paw-paw</td>
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<tr>
<td>Pineapple</td>
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<tr>
<td>Grapes or berries</td>
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<tr>
<td>Melon (e.g. watermelon, rockmelon, honeydew melon)</td>
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</table>

### Vitamin and Mineral Supplements

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Never, or less than once a month</th>
<th>1-3 times per month</th>
<th>Once per week</th>
<th>2-4 times per week</th>
<th>5-8 times per week</th>
<th>Once per day</th>
<th>2-3 times per day</th>
<th>4-5 times per day</th>
<th>6+ times per day</th>
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</thead>
<tbody>
<tr>
<td>Multi vitamin with iron or other minerals</td>
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<tr>
<td>Multi vitamin</td>
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<tr>
<td>Vitamin A</td>
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<td>Vitamin B</td>
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<td>Vitamin C</td>
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<td>Vitamin E</td>
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<tr>
<td>B-carotene</td>
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<tr>
<td>Calcium</td>
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<tr>
<td>Folic Acid/Folate</td>
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<td>Iron</td>
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<td>Zinc</td>
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</tbody>
</table>
Section Two

1. What type of milk do you usually consume?
   - Whole
   - Low/reduced fat
   - Skim
   - Evaporated or sweetened condensed
   - None of the above
   - Don’t know

2. How often do you use any of the following products?

<table>
<thead>
<tr>
<th>Product</th>
<th>Never/Rarely</th>
<th>Sometimes</th>
<th>Usually</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light cream</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sour light cream</td>
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<tr>
<td>Low/reduced fat ice-cream</td>
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<td></td>
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<tr>
<td>Low/reduced fat cheddar-type cheese</td>
<td></td>
<td></td>
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<tr>
<td>Low/reduced oil salad dressing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low/reduced fat spreads</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

3. How often is the meat you eat trimmed of fat either before or after cooking?
   - Never/Rarely
   - Sometimes
   - Usually
   - Don’t eat meat

4. How many serves of vegetables do you usually eat each day? (a 'serve' = 1/2 cup cooked vegetables or 1 cup of salad vegetables)
   - 1 serve or less
   - 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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6. When cooking, which of the following oils/fats do you use?

- Olive oil
- Canola oil
- Vegetable oil
- Butter
- Margarine
- Dairy blend
- Lard or dripping

7. How often do you add salt to your food after it is cooked. Is it never, rarely, sometimes or usually?

- Never/rarely
- Sometimes
- Usually

8. How often is salt added to your food during cooking. Is it never, rarely, sometimes or usually?

- Never/rarely
- Sometimes
- Usually
- Don't know

Please enclose this questionnaire in the folder and reply-paid, self-addressed envelope provided.

Thank you for your co-operation.
## Appendix T – Categories for Food Items Assessed in the Food Frequency Questionnaire based on the AHS 2011-2013 Classification System

<table>
<thead>
<tr>
<th>Category</th>
<th>Two-digit Major Food Groups</th>
<th>Food Frequency Questionnaire items</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain &amp; Cereal</td>
<td>Cereals and cereal products</td>
<td>“white bread, toast or rolls”, “wholemeal/mixed grain bread, toast or rolls”, “English muffin, bagel and crumpet”, “dry or savoury biscuits, crispbread, crackers”, “muesli”, “cooked porridge”, “breakfast cereal”, “rice (including white or brown)”, “pasta (including filled), noodles”</td>
</tr>
<tr>
<td>Meat &amp; Meat Alternatives</td>
<td>Fish and seafood products and dishes</td>
<td>“canned tuna, salmon, sardines”, “fish – steamed, baked, grilled”, “other seafood (eg, prawns)”</td>
</tr>
<tr>
<td></td>
<td>Egg products and dishes</td>
<td>“egg”</td>
</tr>
<tr>
<td></td>
<td>Meat, poultry and game products and dishes</td>
<td>“mince dishes (eg rissoles, meat loaf)”, “mixed dishes with beef, veal (eg, casserole, stir-fry)”, “beef, veal – roast, chop or steak”, “mixed dishes with lamb (eg, casserole, stir-fry)”, “lamb – roast, chop or steak”, “mixed dishes with pork (eg, casserole, stir-fry)”, “pork – roast, chop or steak”, “liver (including pate)”, “mixed dishes with chicken, turkey, duck (eg, casserole, stir-fry)”, “chicken, turkey, duck – roast, steamed, BBQ”</td>
</tr>
<tr>
<td></td>
<td>Seed and nut products and dishes</td>
<td>“nuts”, “peanut butter, other nut spreads”</td>
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<tr>
<td></td>
<td>Dairy and meat substitutes</td>
<td>“soybeans or tofu”</td>
</tr>
<tr>
<td>Fruit</td>
<td>Fruit products and dishes</td>
<td>“apple or pear”, “orange, mandarin or grapefruit”, “banana”, “peach, nectarine, plum or apricot”, “mango or paw-paw”, “pineapple”, “grapes or berries”, “melon (eg, watermelon, rockmelon, honeydew melon)”</td>
</tr>
<tr>
<td>Appendices</td>
<td>1. Low-Fat Dairy</td>
<td>2. High-Fat Dairy</td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>---------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------</td>
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<tr>
<td>Milk products and dishes (dairy milk)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>“milk as a drink”, “milk on breakfast cereals”, “milk in hot beverages (eg, in coffee, tea)”</td>
<td>“cream or sour cream”</td>
</tr>
<tr>
<td>Milk products and dishes (yoghurt)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>“yoghurt, plain or flavoured (including fromage frais)”</td>
<td>“cheddar and other cheeses”, “cottage or ricotta cheese”</td>
</tr>
<tr>
<td>Milk products and dishes (cheese)&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td>“ice-cream”</td>
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<tr>
<td>Milk products and dishes (cream)&lt;sup&gt;1&lt;/sup&gt;</td>
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<tr>
<td>Milk products and dishes (frozen milk products)&lt;sup&gt;1&lt;/sup&gt;</td>
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<tr>
<td>Milk products and dishes (flavoured milks and milkshakes)&lt;sup&gt;1&lt;/sup&gt;</td>
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<tr>
<td>Meat, poultry and game products and dishes</td>
<td>“sausage, frankfurter”, “bacon”, “ham”, “luncheon meats, salami”, “hamburger”</td>
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<tr>
<td>Fish and seafood products and dishes</td>
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<tr>
<td>Vegetable products and dishes</td>
<td>“fish – fried”</td>
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<tr>
<td>Cereal based products and dishes</td>
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<tr>
<td>Savoury sauces and condiments</td>
<td>“oil and vinegar dressing”, “mayonnaise or other creamy dressing”</td>
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<tr>
<td>Snack foods</td>
<td>“potato chips, corn chips, Twisties, etc.”</td>
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<tr>
<td>Sugar products and dishes</td>
<td>“jam, marmalade, syrup or honey”</td>
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<tr>
<td>Confectionary and cereal/nut/fruit/seed bars</td>
<td>“chocolate (including chocolate bars, eg Mars bars)”, “other confectionary”</td>
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<tr>
<td>Miscellaneous</td>
<td>“Vegemite, Marmite, Promite”</td>
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<tr>
<td>Alcoholic beverages</td>
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</tr>
<tr>
<td>Alcoholic beverages</td>
<td>“beer – low alcohol”, “beer – ordinary”, “red wine”, “white wine or champagne/sparkling wine”, “wine cooler”, “sherry or port”, “spirits, liqueurs”</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> Three-digit sub-major food group

<sup>2</sup> Products flagged as discretionary based on the Australian Health Survey – Discretionary Food List (ABS, 2013)