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**Exploring the Link Between Sweet Taste  
and Fat (Creaminess) Perception,  
Dietary Intake and Metabolic Health  
in Women**

A thesis presented in partial fulfilment of the requirements  
for the degree of Doctor of Philosophy in Nutritional Sciences  
at Massey University, Auckland, New Zealand

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## Abstract

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Taste perception plays an important role in dietary choice and intake. There is a significant link between the current obesogenic food environment of ubiquitously available, highly palatable, sugar- and fat-rich foods and adverse metabolic health outcomes. Therefore, it is important to understand the nature of the link between sweet and fat taste perception and dietary intake. Using a multi-disciplinary approach employing sensory science, dietary assessment methods and metabolic health and endocrine analyses, this thesis investigated the relationship between sweet taste and fat (creaminess) perception, dietary intake and metabolic health in women to understand factors contributing or leading to obesity.

The experimental study in Chapter 3 investigated the relationship between four different psychophysical measurements of sweet taste perception and explored which measurements of sweet taste perception relate to sweet food intake. An interesting finding of this chapter was the dose-dependent change in the relationship between sweet taste intensity and hedonic liking, which illustrated that sweet hedonic liking was dependent on the magnitude of sweetness experienced. Importantly, this experimental study showed for the first time a clear dose-dependent link between a lower perceived sweet taste intensity and higher sweet hedonic liking and increased intakes of total energy and carbohydrate (starch, total sugar).

Chapter 4 assessed whether sweet taste and fat (creaminess) perception differ across ethnic groups with known differences in metabolic disease and obesity risk (New Zealand European, Māori, Pacific) and across body composition groups based on body mass index and body fat. Furthermore, this chapter explored whether there is a link between taste perception and metabolic and endocrine biomarkers associated with adiposity and appetite. The overall findings showed no significant differences in sweet taste and fat (creaminess) perception between ethnic groups or body composition groups. Further, no robust links between sweet taste and fat (creaminess) perception and metabolic and endocrine biomarkers were found.

The study described in Chapter 5 explored the links between dietary patterns, body composition, macronutrient intakes and metabolic and endocrine biomarkers of adiposity and appetite. Higher intakes of the 'refined and processed' dietary pattern was linked with higher total energy and percentage carbohydrate (starch, total sugar) intakes and higher body composition measurements (e.g., body mass index, body fat). Furthermore, higher intakes of the 'refined and processed' dietary pattern was linked with higher circulating levels of leptin and insulin and lower levels of ghrelin. Together these findings indicated a diet-induced metabolic dysregulation in women with higher intakes of the 'refined and processed' dietary pattern.

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The research study in Chapter 6 investigated whether body composition, dietary intake and metabolic and endocrine biomarkers differ between women with distinct patterns of sweet and fat (creaminess) hedonic liking. The overall results showed that higher hedonic liking for sweet and fat tastes are linked with increased intakes of sweet and fatty tasting food groups and dietary patterns such as the 'refined and processed' and 'fats and meat' patterns.

Taken together, the experimental studies described in this thesis provide evidence in support of a clear link between sweet taste and fat (creaminess) perception and dietary intake, particularly the intake of foods and dietary patterns characteristic of an individual's taste phenotype. We also found that sweet taste and fat (creaminess) perceptions were not directly linked with body composition, metabolic biomarkers or endocrine regulators in this group of healthy, pre-menopausal women. Furthermore, higher intakes of the 'refined and processed' dietary pattern highlighted a pathway to obesity which appears to be mediated by changes in body composition and key endocrine regulators.

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## List of Abbreviations

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AMDR	Acceptable macronutrient distribution range
BMI	Body mass index
CD36	Cluster of differentiation 36
CI	Confidence interval
CRP	C-reactive protein
DFE	Daily frequency equivalent
DRK	Delayed rectifying potassium
DOHaD	Developmental origins of health and disease
ENaC	Epithelial Na <sup>+</sup> channel
EDTA	Ethylenediaminetetraacetic acid
EXPLORE study	EXAMining Predictors Linking Obesity Related Elements study
FFQ	Food frequency questionnaire
FFA	Free fatty acid
GPCR	G protein coupled receptor
gLMS	General labelled magnitude scale
GLP-1	Glucagon-like peptide-1
HbA1c	Glycosylated haemoglobin
HDL-C	High density lipoprotein cholesterol
HC	Hip circumference
IL-6	Interleukin-6
IL-10	Interleukin-10
INFORMAS	International Network for Food and Obesity/non-communicable diseases Research, Monitoring and Action Support
ISAK	International Society for the Advancement of Kinanthropometry
ICC	Intraclass correlation coefficient
LDL-C	Low density lipoprotein cholesterol
NZE	New Zealand European
OECD	Organisation for Economic Co-operation and Development
PKD2L1	Polycystic kidney disease 2-like 1 protein
PCA	Principal component analysis
SD	Standard deviation
SEM	Standard error of the mean
SF-FFQ	Sweet food-food frequency questionnaire

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TRC	Taste receptor cell
T1	Tertile 1
T2	Tertile 2
T3	Tertile 3
TNF- $\alpha$	Tumour necrosis factor-alpha
T1R1	Type 1 receptor 1
T1R2	Type 1 receptor 2
T1R3	Type 1 receptor 3
VAS	Visual analogue scale
WC	Waist circumference
WHR	Waist to hip ratio
WHO	World Health Organisation

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# Chapter 1

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## *Introduction*

### **1.1 The sense of taste**

Sensory attributes of food such as appearance, smell, taste and texture are important determinants of food choice and intake as these direct us towards particular foods and influence the type and amount consumed (McCrickerd and Forde, 2016). Of these sensory attributes, the relationship between taste and food choice is well established and forms an integral component in the design of foods with preferred taste attributes (Honkanen and Frewer, 2009; Kourouniotis *et al.*, 2016). From an evolutionary point of view, the gustatory system is involved in the detection of different tastes, thereby signalling nutrient-rich foods and foods that may be harmful or toxic (Chandrashekar *et al.*, 2006). In addition to taste detection, the gustatory system evaluates palatability, which is a measure of the hedonic liking associated with the sensory attributes of food (McCrickerd and Forde, 2016). Although hedonic liking does not exclusively determine food intake, many laboratory studies have demonstrated that people tend to eat more of the food they rate as more palatable (Sorensen *et al.*, 2003; Yeomans, 1998). It has also been discussed that repeated exposure to palatable food, especially those with high amounts of sugar and fat, reinforces the pleasure of the food and leads to over-consumption (Berthoud *et al.*, 2011). Therefore, hedonic liking is considered a strong positive reinforcer of food intake.

Emerging data also suggests that the gustatory system is closely linked with the physiological state of the body as several hormones involved in energy homeostasis and appetite regulation were found within the taste buds (Martin *et al.*, 2009; Calvo and Egan, 2015). Consequently, there is growing interest to understand the link between taste perception, dietary intake, energy balance and long-term health (Nasser, 2001; Yeomans, 1998; Donaldson *et al.*, 2009).

### **1.2 The link between obesity and highly palatable, energy-dense food**

Changes in diet and physical activity patterns over the last three decades have paralleled the increased prevalence of overweight and obesity rates worldwide (Popkin, 2006; Hallal *et al.*, 2012; Popkin and Gordon-Larsen, 2004). Currently, New Zealand is the third most obese country in the Organisation for Economic Co-operation and Development (OECD), with nearly two thirds

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of adults either overweight (34%) or obese (32%) (Organisation for Economic Co-operation and Development, 2017; Ministry of Health, 2017). Obesity is a preventable risk factor for many illnesses, including type 2 diabetes, cardiovascular disease, stroke, psychological problems and certain cancers (Guh *et al.*, 2009; Bhaskaran *et al.*, 2014). Furthermore, obesity is associated with a significant social and financial burden to individuals and the healthcare system (Trogon *et al.*, 2008; Withrow and Alter, 2011).

Causes of obesity are multifaceted and involve complex interactions between genetic, metabolic, cultural, environmental, socio-economic and behavioural factors (Albuquerque *et al.*, 2015; Gluckman and Hanson, 2008; Hill *et al.*, 2003). One of the key drivers of the current obesity epidemic is the 'obesogenic' food environment of ubiquitously available, inexpensive, highly palatable and energy-dense foods that are rich in sugar and fat (Swinburn *et al.*, 2011; Vandevijvere and Swinburn, 2014). Evidence from clinical and epidemiological studies show that higher intakes of sugar and fat and certain dietary patterns (e.g., Western, processed) are linked with weight gain, increased adiposity and increased risk of many metabolic illnesses (e.g., type 2 diabetes, cardiovascular disease) (Malik *et al.*, 2006; Te Morenga *et al.*, 2013; Bray and Popkin, 1998; Paradis *et al.*, 2009; Heidemann *et al.*, 2011).

Although obesity rates are increasing worldwide, there are marked differences in obesity rates within countries, where higher rates of obesity are more prevalent in low socio-economic populations and some ethnic groups (Gatineau and Mathrani, 2011; Zilanawala *et al.*, 2015). It has been suggested that the food environment contributes to this socio-economic relationship because healthy foods are often costlier while highly palatable, energy-dense and nutrient-poor foods are more affordable (Drewnowski and Darmon, 2005; Rao *et al.*, 2013; Andrieu *et al.*, 2005). Furthermore, it has been found that differences in diet, physical activity and lifestyle factors may also contribute to the differences in obesity rates observed between populations and ethnic groups (Metcalf *et al.*, 2008; Gatineau and Mathrani, 2011; Ministry of Health, 2006).

Advances in food science techniques have enabled the development of a range of palatable food products made from cheap ingredients and additives (Misra *et al.*, 2017; Floros *et al.*, 2010). Ultra-processed food products that once were exclusively available to high-income countries are now increasingly available to low- and middle-income countries (Monteiro *et al.*, 2013; Baker and Friel, 2016). Furthermore, diets of freshly prepared meals are often replaced with fatty or sugary ready-to-eat food products or fast-food options (Nielsen and Popkin, 2003). Recent research shows that the gustatory system is less able to detect the nutrient content (e.g., the amount of sugar indicated by the perceived sweetness) of highly processed foods compared to

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raw or moderately processed foods (van Dongen *et al.*, 2012; van Langeveld *et al.*, 2017). It is therefore suggested that for highly processed foods, the ability to sense the nutrient content based on taste is rather limited (van Dongen *et al.*, 2012; van Langeveld *et al.*, 2017). This phenomenon has significant implications on food intake and eating behaviour, leading to the over-consumption of energy-dense but nutrient-poor foods, contributing to weight gain and adverse health consequences.

### 1.3 Evaluation of taste perception

Taste receptor cells (TRCs), the functional units of taste detection are present within the taste buds distributed across different papillae of the tongue (Chandrashekar *et al.*, 2006). The TRCs contain many receptors associated with detecting the five basic tastes. Sweet, umami and bitter tastes are detected by G protein coupled receptors (GPCRs), while salty and sour tastes are mediated through ion channels. Furthermore, the recent discovery of many candidate receptors associated with oral fatty acid detection (e.g., cluster of differentiation 36 (CD36), delayed rectifying potassium (DRK) channels) suggests that ‘fat’ may be the sixth taste (Liu *et al.*, 2016; Chalé-Rush *et al.*, 2007).

Sensory evaluation is a scientific discipline that measures and evaluates responses to certain characteristics of food as they are perceived by sight, smell, taste, touch or hearing (Stone *et al.*, 2012). Although traditionally used by sensory scientists to assess consumer responses to foods and beverages (Jellinek, 1985), over the past few decades sensory evaluation has been used in health research to assess the link between taste, dietary intake and obesity (Salbe *et al.*, 2004; Stewart *et al.*, 2010; Low *et al.*, 2016).

Taste perception is commonly characterised by four psychophysical measurements (Lawless and Heymann, 1999). If a tastant (e.g., sucrose) is dissolved at very low concentrations, the difference between the tastant and the background solution (e.g., water) may not be differentiated. However, as the tastant concentration increases, a difference over the background solution is detected. The lowest tastant concentration that can be detected as different from the background solution is defined as detection threshold. As the tastant concentration is increased further, the quality of the taste is recognised. The lowest tastant concentration at which the quality of the taste (e.g., sweet) can be recognised is defined as recognition threshold (Lawless and Heymann, 1999). When the tastant concentration is increased above the threshold level, taste intensity is measured. This is defined as the magnitude of sensation produced by different tastant concentrations. Lastly, hedonic liking is a

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measure of the preference and acceptability of tastants (Lim *et al.*, 2009). Tastant concentrations used to measure taste intensity and hedonic liking are referred to as suprathreshold concentrations, as they are concentrations above threshold levels where the quality of the taste is known (Lawless and Heymann, 1999). In sensory research, measurements of detection threshold, recognition threshold and taste intensity are often considered markers of taste sensitivity, while hedonic liking is considered a marker of preference.

Although people generally like (e.g., sweet) and dislike (e.g., bitter) similar tastes, hedonic liking for a specific tastant concentration varies widely between individuals (Proserpio *et al.*, 2017; Kim *et al.*, 2014). Furthermore, there are inter-individual variations in the concentrations at which tastes are detected and recognised (detection and recognition thresholds) and in the level of intensity produced by a tastant concentration (taste intensity). Differences in these taste perception characteristics can influence dietary intake and eating behaviour (Lampuré *et al.*, 2016; Nasser, 2001). Therefore, it is important to use standardised and valid psychophysical methods to measure taste perception in order to accurately assess biological and physiological links between taste, dietary intake and metabolic health.

## **1.4 Assessment of dietary intake**

Dietary assessment is a comprehensive evaluation of food intake, including the quantity, frequency, patterns and the quality of foods consumed by individuals or groups (Willett, 2013). Dietary intake data obtained from various dietary assessment methods are used to assess relationships between diet and nutrition-related health outcomes (Biro *et al.*, 2002). Dietary assessment of an individual can be conducted using retrospective or prospective methods (Gibson, 2005). Retrospective methods include recalling food intake from the previous day (24-hour diet recall) or collecting information on usual food intake over the previous months or year (diet history or food frequency questionnaire (FFQ)). Prospective methods include maintaining a record of all foods and beverages consumed over a period of time (food record) (Biro *et al.*, 2002). Each dietary assessment method has its own strengths and limitations. Thus, the appropriate dietary assessment method should be selected based on the specific objectives of the study (Biro *et al.*, 2002). For example, food records are used to measure actual food intake, while FFQs can be used to assess usual food intakes or frequency of food intakes (Gibson, 2005).

There are many challenges associated with self-reported dietary assessment data (Subar *et al.*, 2015). For example, 24-hour diet recalls rely on memory and certain foods such as discretionary food items may not be recorded. Furthermore, FFQs have a finite list of foods and show

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respondent bias due to social pressure, while food records are susceptible to modifications in intake during data collection and as a result many foods and beverages may be omitted (Vucic *et al.*, 2009; Gibson, 2005; Subar *et al.*, 2015). Due to these reasons, dietary intake data often suffer from measurement error, which is identified as the difference between the measured value and true value (Subar *et al.*, 2015). Therefore, it is recommended that several dietary assessment methods are used in parallel to obtain dietary data that maximise the strengths of each method. Furthermore, the interpretation of dietary data should be done keeping in mind the limitations of the dietary assessment method (Subar *et al.*, 2015).

## **1.5 The link between sweet and fat taste perception and dietary intake**

Many studies have investigated the link between different psychophysical measurements of sweet taste perception and dietary intake. The overall findings of these studies suggest a positive correlation between hedonic liking of sweet solutions and the frequency of sweet food and refined sugar intake (Holt *et al.*, 2000), preferences for sweet desserts and sugar in tea (Drewnowski *et al.*, 1999) and the sugar content of favourite cereal (Mennella *et al.*, 2011). However, no robust associations have been found between dietary intake and sweet taste thresholds or sweet taste intensity (Low *et al.*, 2016; Cicerale *et al.*, 2012).

Fat taste is an emerging area of interest in sensory research due to the association between excessive intakes of dietary fats and obesity (Bray and Popkin, 1998). Some studies have shown that individuals with lower oleic acid detection thresholds (i.e., more sensitive) had lower energy intakes, consumed less dietary fats and were better at detecting differences in fat content than individuals with higher oleic acid thresholds (i.e., less sensitive) (Stewart *et al.*, 2011a; Stewart *et al.*, 2010). Furthermore, individuals who rated fat containing samples as more intense had lower preferences for high-fat foods and consumed smaller amounts of fast-food compared to individuals who rated the samples as less intense (Martínez-Ruiz *et al.*, 2014). While one study reported a positive correlation between the hedonic liking of fat taste and dairy intake (Shen *et al.*, 2017), others found no relationships between any measurement of fat taste perception and dietary intake (Mela and Sacchetti, 1991; Keast *et al.*, 2014).

Methodological factors associated with taste perception and dietary assessment methods may contribute to the inconsistent findings between studies. Taste thresholds are often used as a measure of taste sensitivity. However, thresholds determine the lowest concentration of a taste that is detected or recognised (Bartoshuk, 1978). It has been suggested that the low tastant

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concentrations associated with taste thresholds relate little to dietary intake. Suprathreshold measures (taste intensity and hedonic liking) are more appropriate when assessing relationships between taste and diet, as suprathreshold tastant concentrations are closer to concentrations found in real-world foods and beverages (Bartoshuk *et al.*, 2005; Bartoshuk *et al.*, 2004a). Therefore, methods evaluating taste sensitivity and hedonic liking require further improvements, refinements and standardisation.

A variety of dietary assessment methods have been used to assess different measures of dietary intake. These include food records, 24-hour diet recalls, FFQs and other types of questionnaires such as food variety surveys (Low *et al.*, 2016; Cicerale *et al.*, 2012; Holt *et al.*, 2000; Keast *et al.*, 2014). These dietary assessment methods may not have accurately captured food intake due to participants modifying their diets during the intervention or dietary methods that depend on memory causing reliability issues leading to over- or under-reporting of data. This illustrates the need to use validated and standardised dietary tools to assess the relationship between sweet and fat taste perception and dietary intake.

## **1.6 The link between sweet and fat taste perception and obesity**

Given the positive link between excess sugar and fat intake and weight gain (Malik *et al.*, 2006; Bray and Popkin, 1998), it is important to understand whether differences in sweet and fat taste perception between normal-weight and overweight/obese individuals influence the type and amount of sweet and fatty foods consumed. Therefore, the first step is to establish whether a relationship between the different psychophysical measures of sweet and fat taste perception and obesity exists. If lower sweet and fat taste sensitivity and/or higher sweet and fat hedonic liking are linked with obesity, then factors that influence sweet and fat taste perception can be identified in order to explore avenues to reduce weight gain and obesity (e.g., specific dietary approaches).

To date there is little agreement whether a relationship between sweet taste perception and obesity exists. Some studies report that overweight/obese individuals have higher sucrose detection thresholds and find sucrose solutions to be less intense compared to normal-weight individuals (Sartor *et al.*, 2011; Ettinger *et al.*, 2012). In contrast, a recent study found that obese individuals have lower sweet recognition thresholds and perceive sweet solutions as more intense compared to normal-weight individuals (Hardikar *et al.*, 2017). However, most studies found no clear link between any sweet taste perception measurement and obesity (Low *et al.*, 2016; Martinez-Cordero *et al.*, 2015; Park *et al.*, 2015; Pepino *et al.*, 2010). The findings of the

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studies assessing the link between fat taste perception and obesity are also inconclusive. For example, some studies have shown that individuals hyposensitive (i.e., less sensitive) to the detection of oleic acid had a higher body mass index (BMI) compared to hypersensitive individuals (Stewart *et al.*, 2011a; Stewart *et al.*, 2010). Furthermore, another study showed that individuals who had higher intensity ratings for linoleic acid had a lower BMI and waist circumference (WC) than those who had lower intensity ratings (Martínez-Ruiz *et al.*, 2014). However, most studies found no clear link between any fat taste perception measurement and obesity (Chevrot *et al.*, 2014; Stewart and Keast, 2012; Salbe *et al.*, 2004; Pepino and Mennella, 2014).

Many methodological factors associated with taste perception measurements and the experimental design of studies can be attributed to the discrepancies in findings between studies. Factors associated with the psychophysical measurements of taste used in different studies (e.g., type of tastants, concentration levels, type of psychophysical measurement, sample presentation method) can introduce variations in measurements and mask true associations. Furthermore, taste intensity and hedonic liking measurements obtained using certain rating scales (e.g., visual analogue scale (VAS)) are not suitable for across-group taste perception comparisons due to labels referring to different experiences (Bartoshuk *et al.*, 2003; Bartoshuk *et al.*, 2004b). The general labelled magnitude scale (gLMS) is considered the most appropriate method to compare across-group sensory perceptions as ratings are performed with reference to the 'strongest imaginable sensation of any kind' experienced by each person (Bartoshuk *et al.*, 2003; Bartoshuk *et al.*, 2004b). In addition, factors associated with the experimental design of studies, including differences in participant characteristics (e.g., age, ethnic group, BMI range), relatively small sample sizes (i.e., not enough statistical power to detect differences in taste perception between BMI groups) and narrow BMI ranges (i.e., does not provide enough variation in the data to explore relationships) can contribute to the inconsistent findings and mask true biological relationships.

Although many studies have investigated the link between sweet and fat taste perception and obesity, as discussed above, the findings of these studies are inconsistent. These inconsistent and contradicting results highlight the need for further investigation in larger cohorts, with well-defined BMI and body fat groups, using established and validated sensory techniques.

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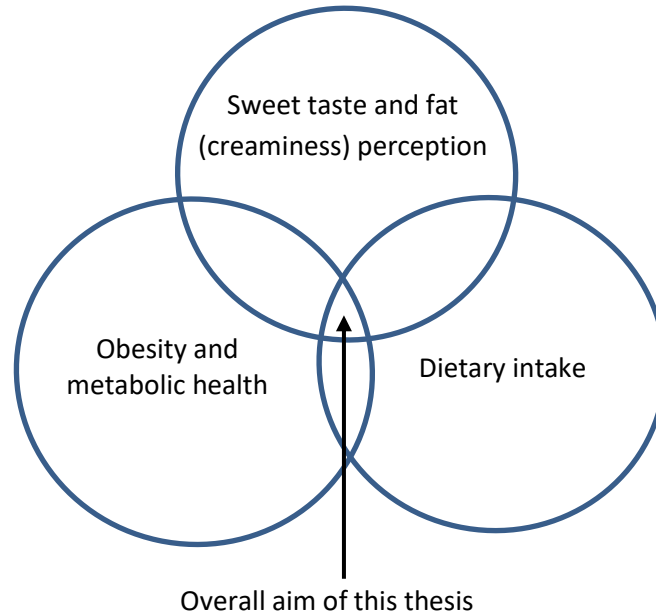
## 1.7 Significance of research

Although taste is considered an important driver of food choice, the inconsistent and contradicting findings discussed above show no clear link between sweet and fat taste perception and dietary intake. A better understanding of how inter-individual variations in sweet and fat taste perception influence dietary intake, can help to explain reasons behind people's food choices and eating habits. If a relationship between sweet and fat taste perception and dietary intake was found, then specific strategies to change taste perception (e.g., specific dietary approaches, targeted reformulation) can be developed to break the cycle of habituation to unhealthy foods. Furthermore, high obesity rates in New Zealand and worldwide are creating major challenges for the individual and escalating demands on the healthcare system both in terms of treatment costs and reduced productive years (The GBD Obesity Collaborators, 2017; Ministry of Health, 2017). Tackling obesity requires a multifaceted approach. A better understanding of how sweet and fat taste perception drives dietary choice and intake will refine future dietary recommendations and open prevention strategies to reduce the intake of highly palatable, energy-dense foods (e.g., tailored dietary guidance and support to make healthy changes in daily eating habits). Furthermore, assessing how different taste characteristics influence dietary intake will provide evidence-based information supporting the need for reformulated food products. The reformulation of food products through reducing sugar and fat content (while maintaining palatability) has the potential to reduce passive over-consumption of energy and improve diet quality (Yeung *et al.*, 2017; Jensen and Sommer, 2017). Policymakers together with the co-operation of the food industry can work to improve the nutritional quality of foods available on the market and provide healthier food options to all population groups (Chauliac and Hercberg, 2012; Hendry *et al.*, 2015).

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## 1.8 Study aim, objectives and hypotheses

### 1.8.1 Study aim



The overall aim of this thesis was to advance our understanding of the relationship between sweet taste and fat (creaminess) perception and dietary intake, and, how this may influence body composition in women in order to understand factors contributing or leading to obesity.

### 1.8.2 Study objectives and hypotheses

The specific objectives of this thesis were to:

1. Explore the relationship between four commonly used measurements of sweet taste perception (detection threshold, recognition threshold, sweet taste intensity, sweet hedonic liking) and investigate which measurements of sweet taste perception are linked with sweet food intake. (Chapter 3)
2. Evaluate differences in sweet taste and fat (creaminess) perception across different ethnic groups with known differences in metabolic disease and obesity risk (New Zealand European (NZE), Māori, Pacific) and across well-defined body composition groups (BMI, body fat). Furthermore, to explore the relationship between sweet taste and fat (creaminess) perception and key metabolic and endocrine biomarkers of adiposity and appetite. (Chapter 4).

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3. Identify dietary patterns in women from different ethnic groups with known differences in metabolic disease and obesity risk and to investigate the link between these dietary patterns, body composition, macronutrient intakes and metabolic and endocrine biomarkers of adiposity and appetite. (Chapter 5)
  4. Identify and characterise patterns of sweet and fat (creaminess) hedonic liking and to investigate whether body composition, dietary intake and metabolic and endocrine biomarkers of adiposity and appetite differ between women with different patterns of hedonic liking. (Chapter 6)

The specific hypotheses of the thesis were:

1. Lower sweet taste perception (i.e., higher detection threshold, higher recognition threshold and/or lower perceived sweet taste intensity) and/or higher hedonic liking are associated with increased intakes of sweet tasting foods. (Chapter 3)
2. Differences in sweet taste intensity and hedonic liking and/or fat (creaminess) taste intensity and hedonic liking may be explained by body fat profiles and metabolic and endocrine biomarkers of adiposity and appetite. (Chapter 4)
3. Distinct dietary patterns may relate to population groups with different metabolic disease and obesity risk profiles, body composition measurements, macronutrient intakes and metabolic and endocrine biomarkers of adiposity and appetite. (Chapter 5)
4. Body composition measurements, dietary intake and metabolic and endocrine biomarkers of adiposity and appetite will differ between women with different patterns of sweet and fat (creaminess) hedonic liking. (Chapter 6)

Data from two experimental studies of cross-sectional design (sweet taste study and women's EXamining Predictors Linking Obesity Related Elements (EXPLORE) study) were used to investigate the above objectives. The assessment of sweet taste and fat (creaminess) perception was chosen due to the established link between excess sugar and fat intake and weight gain and obesity (Te Morenga *et al.*, 2013; Bray and Popkin, 1998). Both experimental studies recruited women as recent data show a significant rise in obesity in New Zealand women between the period of 2006 to 2016. Furthermore, currently obesity rates are higher in New Zealand women than men (Ministry of Health, 2017). The long-term health impact of increased adiposity in women of child-bearing age is associated with adverse maternal and neonatal health outcomes,

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and increased obesity risk for the offspring (Marchi *et al.*, 2015; Crane *et al.*, 2009). Therefore, it is important to understand factors that increase the risk of obesity in women of reproductive age in order to slow down the progression of obesity.

The first cross-sectional study (sweet taste study) investigated objective one in a group of 44 NZE women between the ages of 20–40 years. The four sweet taste perception measurements chosen for this study (detection threshold, recognition threshold, sweet taste intensity, sweet hedonic liking) are commonly used in taste research and characterise different components of sweet taste. Repeatability was tested over four repeated sessions to evaluate and identify the most consistent sweet taste perception measurements. Three different dietary measurements (weighed food record, sweet food focused FFQ, sweet beverage liking questionnaire) were used to capture different aspects of sweet food intake and liking.

The second cross-sectional study (Women’s EXPLORE study) was designed to explore the metabolic disease risks and predictive factors associated with different body fat profiles of 16–45 year old NZE, Māori and Pacific women (Kruger *et al.*, 2015). These ethnic groups were chosen due to the known differences in metabolic disease risk and the disproportionate obesity rates. As reported in the latest New Zealand Health Survey, Māori and Pacific women have a higher risk of metabolic disease and obesity (52% and 73% obese respectively), while NZE women have a moderate risk of metabolic disease and obesity (32% obese) (Ministry of Health, 2017). Objectives two to four were investigated using data obtained from the larger EXPLORE study involving 408 women. Sweet taste perception was measured by rating the sweet taste intensity and sweet hedonic liking of five sweet samples with varying levels of sucrose. Fat taste perception was measured by rating the creaminess intensity and creaminess hedonic liking of five milk samples with varying levels of fat. As fat taste perception has many components, including mouthfeel, texture and aroma, we used ‘creaminess’ to assess the overall experience of fat taste produced by the tastant (i.e., milk samples). Body composition measurements included body weight, BMI, body fat and anthropometric measurements. Furthermore, metabolic biomarkers and endocrine regulators associated with appetite regulation, glucose homeostasis, lipid profile and inflammation were also analysed. A 220-item semi-quantitative FFQ was used to assess dietary intake and to derive dietary patterns of the study population.

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## **1.9 Structure of thesis**

This thesis begins with a comprehensive review of the literature around the three main topics of the aim; sweet and fat taste perception, dietary intake and obesity and metabolic health (Chapter 2). The subsequent four chapters of the thesis are the experimental chapters outlining the main findings of the two cross-sectional studies. The main findings of the relationship between sweet taste perception measurements and sweet food intake is reported in Chapter 3. Chapter 4 assessed whether sweet taste and fat (creaminess) perception differed between ethnic groups and well-defined body composition groups. In Chapter 5, the link between dietary patterns, body composition, macronutrient intakes and metabolic biomarkers was investigated. Chapter 6 investigated whether body composition, dietary intake and metabolic biomarkers differed between patterns of sweet and fat (creaminess) hedonic liking. Chapter 7, the final discussion, brings together the main findings of the four experimental chapters of this thesis. This chapter also includes recommendations for future studies based on the main findings and limitations of the four experimental chapters.

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## Chapter 2

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### *Literature Review*

#### **2.1 Introduction**

Sensory attributes such as taste, smell, mouthfeel and texture influence food intake and eating habits (McCrickerd and Forde, 2016). Of these attributes, the sense of taste is considered an important contributor of food choice, as the decision to choose one type of food over another often depends on taste (Kourouniotis *et al.*, 2016; Sorensen *et al.*, 2003). The gustatory system is responsible for detecting different tastes and evaluating the palatability of food, thereby acting as a nutrient sensing system (Yeomans, 1998; Chandrashekar *et al.*, 2006). The increased availability and over-consumption of energy-dense foods that are high in added sugar and fat have contributed to the current ‘obesogenic’ food environment favouring excess energy availability and weight gain (Swinburn *et al.*, 2011; Nielsen and Popkin, 2003). The positive hedonic pressures associated with the palatability of sugar- and fat-rich food is considered a key driving force that promotes over-consumption of food and a positive energy balance (Sorensen *et al.*, 2003; Drewnowski, 1997a). Although individuals generally like and dislike similar tastes, there are significant inter-individual variations in how sweet and fat tastes are detected, perceived and liked (Low *et al.*, 2016; Asao *et al.*, 2015; Liang *et al.*, 2012; Tucker and Mattes, 2013). These variations in sweet and fat taste perception can influence the type and amount of sweet and fatty foods consumed, thus impact long-term health (Keast *et al.*, 2014; Deglaire *et al.*, 2015).

This literature review begins with a description of the anatomy of the gustatory system and the detection of different tastes. Sweet and fat taste will be the focus of this review due to the positive association between excess sugar and fat intake and adverse metabolic health (Te Morenga *et al.*, 2013; Bray and Popkin, 1998). This review will next provide a concise description of the current obesity epidemic including the main contributors and health consequences of obesity. An emphasis will be placed on examining the current food environment which promotes the intake of highly palatable sugar- and fat-rich foods. Next, an overview of the most commonly used sensory evaluation methods and the challenges associated with measuring taste perception will be discussed. The last two sections of this review will examine the current knowledge of the associations between sweet and fat taste perception, dietary intake and obesity. The literature review will end with an overall summary highlighting the knowledge gaps addressed in each of the four experimental chapters of this thesis.

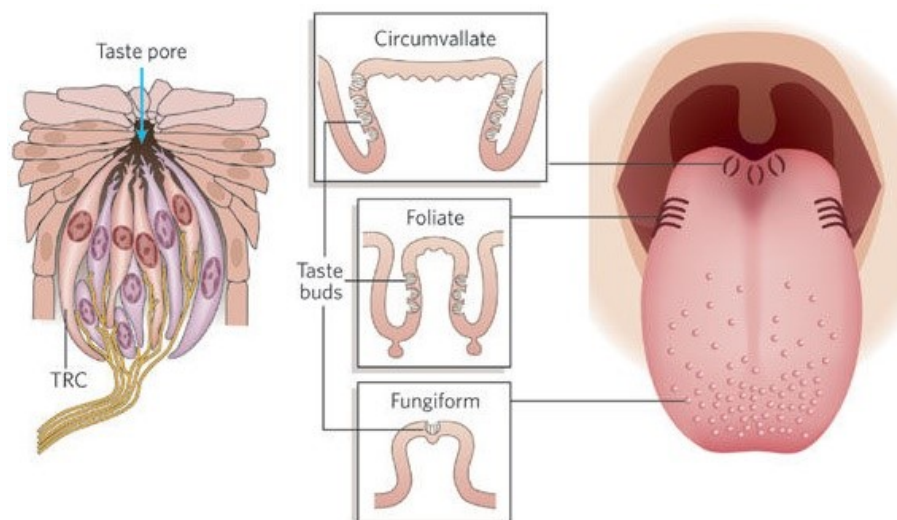
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## 2.2 The sense of taste

The sense of taste plays an important role in food choice and intake (Drewnowski, 1997a). Taste in general refers to the combination of all sensory perceptions associated with the presence of food in the mouth, including taste and olfaction. More specifically, taste perception refers to the detection of the five basic tastes by the gustatory system (Chandrashekar *et al.*, 2006). From an evolutionary point of view, taste perception evaluates the nutritional value of food (i.e., sweet, salty and umami tastes) and prevents the ingestion of toxic or harmful substances (i.e., bitter and sour tastes), thus leading to specific eating responses (Simon *et al.*, 2006; Chandrashekar *et al.*, 2006). In addition, the gustatory system evaluates palatability, defined as the positive hedonic liking associated with the sensory characteristics of food (Yeomans, 1998).

### 2.2.1 The anatomy of the gustatory system

The gustatory system consists of 50–100 taste cells grouped into specialised structures called taste buds mainly found within the fungiform, foliate and circumvallate papillae of the tongue (Figure 2.1) (Chandrashekar *et al.*, 2006). On the apical side taste buds are in contact with the oral cavity through a narrow opening called the taste pore. On the basolateral side taste buds synapse with the afferent gustatory nerve fibres which transmit taste information to the brain (e.g., chorda tympani and branches of the facial nerve) (Simon *et al.*, 2006).



**Figure 2.1 Taste buds and taste receptor cells found within the papillae of the tongue**

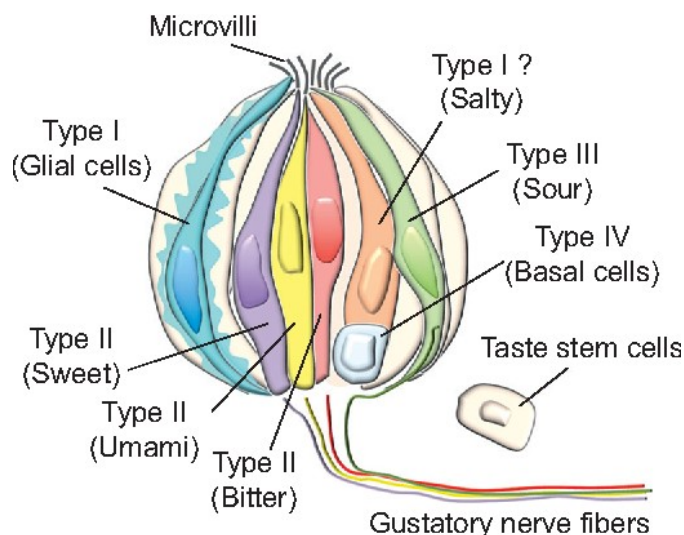
TRC: taste receptor cell.

Reprinted from Nature. Chandrashekar, *et al.*, 2006, The receptors and cells for mammalian taste, with permission from Macmillan Publishers Ltd.

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Each taste bud has four main types of taste cells with distinct functions (Figure 2.2) (Besnard *et al.*, 2016; Shigemura and Ninomiya, 2016). Type I taste cells also known as ‘glial-like cells’ are the most abundant cells in the taste buds. Type I taste cells play a role in limiting the spread of synaptic transmission by preventing changes in ion concentrations reaching other regions of the taste bud (Bartel *et al.*, 2006). Furthermore, type 1 cells may also be involved in the detection of salty taste (Vandenbeuch *et al.*, 2008). Type II taste cells are the basic TRCs associated with the detection of sweet, bitter and umami tastes (Boughter Jr *et al.*, 1997; Yang *et al.*, 2000).

Type III taste cells also known as ‘pre-synaptic cells’ form synaptic junctions with the afferent gustatory nerve fibres and transmit taste information to the brain (Yang *et al.*, 2000). In addition, type III taste cells may also be involved in the detection of sour taste (Huang *et al.*, 2008b). Lastly, type IV basal precursor cells are the dividing progenitor cells responsible for the renewal of taste cells that differentiate into other cell types (Figure 2.2) (Martin *et al.*, 2009).



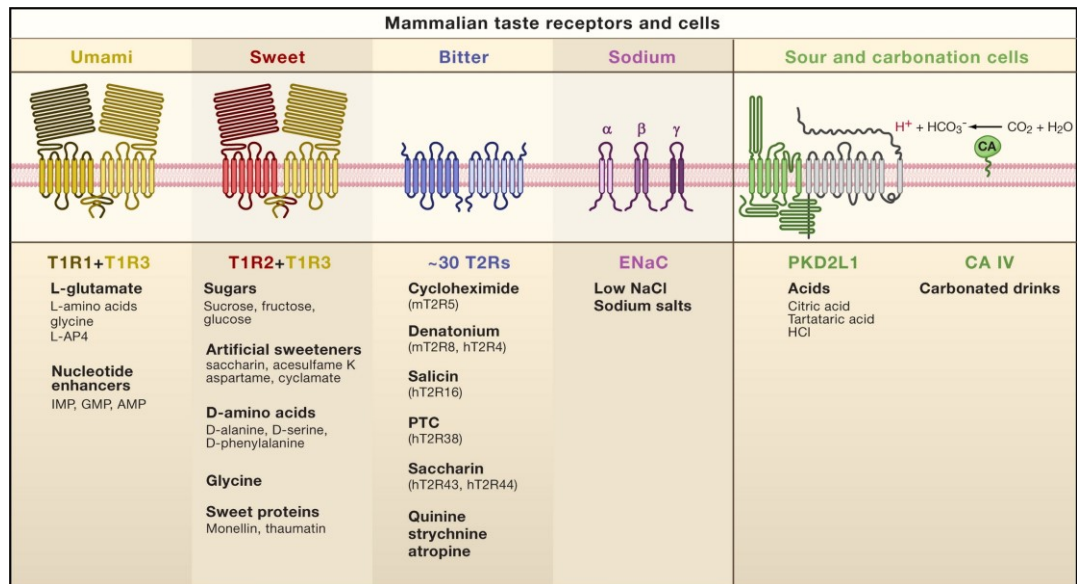
**Figure 2.2 Functional characteristics of the four main cell types of taste buds**

Reprinted from International Review of Cell and Molecular Biology. Shigemura and Ninomiya, 2016, Recent advances in molecular mechanisms of taste signaling and modifying from Elsevier Academic Press Inc.

Similar to other cells in the body, taste cells undergo a renewal process. Taste buds arise from the basal precursor cells of the epithelium and have a lifespan of 8–12 days and a turnover of approximately 10% each day (Beidler and Smallman, 1965; Hevezi *et al.*, 2009). In addition to the genes associated with the cell renewal process, taste buds contain several other genes associated with neuronal signalling, taste detection, endocrine regulation and the immune system. This suggests that taste buds are involved in many important physiological and metabolic processes (Hevezi *et al.*, 2009).

## 2.2.2 Detection of taste

To date the five known basic taste qualities are sweet, sour, salty, bitter and umami (Chandrashekar *et al.*, 2006). Although not considered a basic taste, fat taste has gained interest over the last few decades due to the positive link between excess fat intake and the development of obesity and other chronic illnesses (Liu *et al.*, 2016). Taste receptors involved in the detection of the five basic tastes are shown in Figure 2.3.



**Figure 2.3 Taste receptors associated with the detection of the five basic taste qualities**

Umami, sweet and bitter tastes are detected by receptors of the G protein coupled receptor family, while salty and sour tastes are detected by specialised ion channels.

T1R1: type 1 receptor 1, T1R2: type 1 receptor 2, T1R3: type 1 receptor 3, T2Rs: type 2 receptors, ENaC: epithelial Na<sup>+</sup> channel, PKD2L1: polycystic kidney disease 2-like 1 protein, CA IV: carbonic anhydrase IV. Reprinted from Cell. Yarmolinsky *et al.*, 2009, Common sense about taste: From mammals to insects, 2009, with permission from Elsevier.

As shown in Figure 2.3, sweet, umami and bitter tastes are detected by GPCRs located within the type II taste cells (Martin *et al.*, 2009). Three subunits of the GPCR type 1 receptor family heterodimerise to form the sweet receptor (type 1 receptor 2 (T1R2) + type 1 receptor 3 (T1R3)) and the umami receptor (type 1 receptor 1 (T1R1) + type 1 receptor 3 (T1R3)) (Li *et al.*, 2002). The term umami was coined by Ikeda in 1908 to describe the unique broth-like sensation elicited by seaweed, dried fish and mushrooms (Yamaguchi, 1991). In humans, umami taste is strongly stimulated only by L-glutamate (monosodium glutamate) and L-aspartate, whereas mice display robust attraction and neural responses to majority of L-amino acids (Nelson *et al.*, 2002; Yarmolinsky *et al.*, 2009).

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Possibly due to the challenge of identifying multiple bitter compounds at very low concentrations, bitter taste is mediated through a separate GPCR type 2 receptor family consisting of 25–30 receptors (Meyerhof, 2005). Significant variations in sensitivity to bitter tastants is achieved through several distinct receptor polymorphisms of the type 2 receptors (Chandrashekar *et al.*, 2006). Sour and salty tastes are modulated by the direct entry of Na<sup>+</sup> and H<sup>+</sup> ions through taste pores (Chandrashekar *et al.*, 2006). Sour taste of acidic compounds is elicited by activating type III taste cells within the taste buds. Although the exact mechanism is not completely known, studies suggest that polycystic kidney disease 2-like 1 protein (PKD2L1) is involved in sour taste detection (Figure 2.3) (Huang *et al.*, 2008a; Huang *et al.*, 2006). The molecular signalling associated with salty taste is also not known. Research suggests that Na<sup>+</sup> ions can enter via amiloride-sensitive epithelial Na<sup>+</sup> channels (ENaCs) expressed within type I taste cells (Figure 2.3) (Chandrashekar *et al.*, 2010).

### **2.2.3 Sweet taste perception**

The recognition of different sweet compounds is achieved through multiple ligand binding sites located on the T1R2+T1R3 sweet receptor (Temussi, 2007; Roper, 2007). The T1R2+T1R3 receptor is responsible for the identification of a diverse range of sweet compounds, including natural sugars (e.g., glucose, fructose, sucrose), sugar alcohols (e.g., xylitol, erythritol, sorbitol), low caloric natural sweeteners (e.g., Stevia) and artificial sweeteners (e.g., aspartame, saccharin, sucralose) (Yarmolinsky *et al.*, 2009; Masuda *et al.*, 2012). Functional studies have shown that all three domains of the T1R2+T1R3 receptor, the extracellular N-terminal venus-flytrap domain, the seven transmembrane spanning domain and the cysteine-rich domain are involved in detecting various sweet stimuli (Li, 2009). For example, the T1R2 N-terminal venus-flytrap domain recognises many artificial sweeteners such as aspartame, neotame and saccharin (Masuda *et al.*, 2012; Li, 2009), while the T1R3 transmembrane domain recognises cyclamate and lactisole (Li, 2009).

### **2.2.4 Fat taste perception**

The recent discovery of putative fatty acid receptors in the taste buds suggest that the gustatory system may play a role in oral fatty acid detection (Mela, 1988; Ramirez, 1994). Furthermore, recent research shows that humans can detect free fatty acids (FFAs) varying in length and saturation when olfactory and textural cues are masked (Chalé-Rush *et al.*, 2007; Stewart *et al.*, 2010). To detect taste, the tastant must be soluble in saliva, but triglyceride, the predominant form of fat in foods is not soluble in saliva. It was first shown in rodent studies that lingual lipase

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produced by the salivary glands play a role in hydrolysing triglycerides into FFAs (Kawai and Fushiki, 2003). Human studies have also shown that lingual lipase generates FFAs from food and that the FFA concentrations are sufficient to initiate signalling pathways within the gustatory system (Kulkarni and Mattes, 2013; Pepino *et al.*, 2012). Several candidate receptors involved in fatty acid detection have been identified. These include the CD36, DRK channels and GPCRs 120 and 40, which recognise a variety of FFAs varying in length and saturation (e.g., medium and long chain fatty acids, polyunsaturated fatty acids) (Laugerette *et al.*, 2005; Galindo *et al.*, 2012; Besnard *et al.*, 2016). However, the sensory mechanisms involved in fat perception are complex as fat perception is a multimodal integration of olfactory, gustatory and somatosensory cues such as texture, aroma, lubricity, oiliness and creaminess (Feron and Poette, 2013; Khan and Besnard, 2009).

### **2.3 Obesity and metabolic health**

The World Health Organisation (WHO) defines overweight and obesity as ‘abnormal or excessive fat accumulation that may impair health’ (World Health Organisation, 2000). Body mass index is a simple tool used to classify overweight and obesity calculated by weight in kilograms divided by the square of the height in metres. The basis of BMI was developed by Adolphe Quetelet in 1832 (Eknoyan, 2008). He concluded that other than the growth spurts after birth and at puberty, weight increases as the square of the height. This was known as the Quetelet index until it was termed as BMI in 1972 (Eknoyan, 2008). According to the WHO, the adult BMI classifications are; normal-weight (18.5–24.9 kg/m<sup>2</sup>), overweight (25–29.9 kg/m<sup>2</sup>) and obese (≥30 kg/m<sup>2</sup>) (World Health Organisation, 2000). Within the obese category there are three further subclasses; obese class I (30–34.9 kg/m<sup>2</sup>), obese class II (35–39.9 kg/m<sup>2</sup>) and obese class III (≥40 kg/m<sup>2</sup>) (World Health Organisation, 2000).

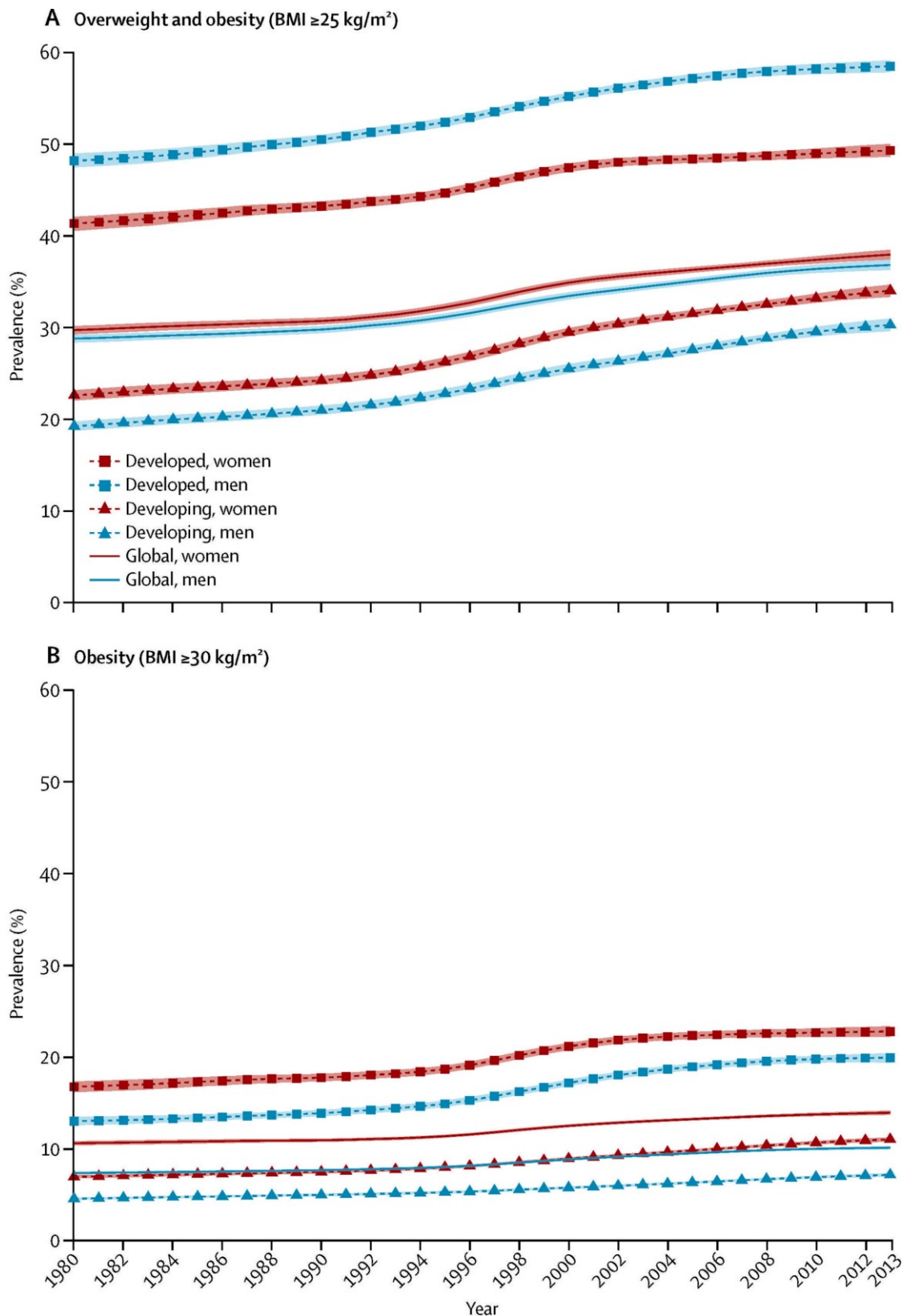
Body mass index is a non-invasive tool used in research and clinical settings as a proxy for the amount of body fat mass (Gallagher *et al.*, 1996). Furthermore, BMI can be used as a surrogate measure of adiposity-related health risks, as it correlates positively with WC, waist to hip circumference ratio and biomarkers associated with chronic illnesses such as type 2 diabetes and cardiovascular disease (Qiao and Nyamdorj, 2009; de Koning *et al.*, 2007; Pischon *et al.*, 2008). Body mass index has many limitations. For example, BMI approximates body fat mass, it does not take into account body size and it provides no information about the distribution of body fat mass (Nevill *et al.*, 2006; Okorodudu *et al.*, 2010). Furthermore, BMI cut-offs do not reflect gender-related differences or age-related changes in body composition (Jackson *et al.*, 2002; Kyle *et al.*, 2001). There is growing debate whether ethnic group-specific BMI cut-offs

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need to be generated due to the differences in body composition (fat and muscle mass), body fat distribution and metabolic disease risks between ethnic groups (Rush *et al.*, 2009; Misra and Khurana, 2011). For example, South Asians have higher body fat and develop type 2 diabetes at lower BMI ranges than Europeans of the same age, gender and BMI, suggesting the need to have lower cut-offs for some ethnic groups (Lear *et al.*, 2007; Chiu *et al.*, 2011). Due to these limitations, in addition to BMI, anthropometric measurements (i.e., WC, waist to hip circumference ratio) and metabolic biomarkers (i.e., glucose, glycosylated haemoglobin (HbA1c), lipid profile) are commonly used as diagnostic markers of metabolic health risks (Gomez-Ambrosi *et al.*, 2012).

### **2.3.1 Prevalence of overweight and obesity**

Over the last 30 years, the prevalence of overweight and obesity has increased in adults and children in both developed and developing countries (Figure 2.4) (Ng *et al.*, 2014; Abarca-Gómez *et al.*, 2017). Between the periods of 1980 to 2013, the number of overweight and obese adults worldwide increased from 857 million to 2.1 billion. Consequently, approximately one in three individuals worldwide are overweight or obese (Abarca-Gómez *et al.*, 2017). Worldwide, the proportion of overweight and obese men increased from 28.8% in 1980 to 36.9% in 2013 and overweight and obese women increased from 29.8% to 38.0% during the same period (Ng *et al.*, 2014). Data show that in developed countries overweight and obesity rates are higher in men compared to women and in developing countries overweight and obesity rates are higher in women than men (Figure 2.4) (Ng *et al.*, 2014).



**Figure 2.4 Overweight and obesity rates in adults over the period of 1980–2013**

Reprinted from The Lancet. Ng, *et al.*, 2014, Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: A systematic analysis for the Global Burden of Disease Study 2013, with permission from Elsevier.

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Worldwide overweight and obesity rates in children and adolescents have also increased over the past three decades (Ng *et al.*, 2014; Abarca-Gómez *et al.*, 2017). Although it was once considered a problem of high-income countries, the prevalence of overweight and obesity in children has significantly increased in low- and middle-income countries (World Health Organisation, 2000). As estimated in 2013, 23.8% of boys and 22.6% of girls were overweight or obese in developed countries and 12.9% of boys and 13.4% of girls were overweight or obese in developing countries (Ng *et al.*, 2014).

Although the prevalence of overweight and obesity in children and adults is increasing throughout the world, the distribution varies greatly within countries (Ng *et al.*, 2014; Zilanawala *et al.*, 2015). Within countries there is evidence of obesity affecting certain ethnic groups and low socio-economic groups. For example, in the United States, African American and Mexican American women have a higher prevalence of obesity than Caucasian women or men (Flegal *et al.*, 2016). Similarly, in the United Kingdom, obesity rates vary substantially between ethnic groups for both adults and children (Zilanawala *et al.*, 2015; Gatineau and Mathrani, 2011). For example, one study found that Black African and Black Caribbean children have higher odds of being overweight and obese in comparison to Caucasian children (Zilanawala *et al.*, 2015). Differences in obesity rates between different ethnic groups may be attributed to several reasons, including differences in lifestyle, culture, physical activity, diet, attitudes towards health and the socio-economic environment (Gatineau and Mathrani, 2011; Zilanawala *et al.*, 2015).

### **2.3.2 Overweight and obesity rates in New Zealand**

Similar to the prevalence worldwide, overweight and obesity rates in New Zealand adults have increased significantly over the last few decades. Currently, one in three adults are obese and a further one in three are overweight (Table 2.1) (Ministry of Health, 2017). Overall, overweight rates are higher in men compared to women, while obesity rates are higher in women than men (Table 2.1). Overweight and obesity rates vary between ethnic groups. Overall, obesity rates are highest in Māori and Pacific adults and lowest in European/other and Asian adults, whereas overweight rates are higher in European/other and Asian adults compared to Māori and Pacific adults (Ministry of Health, 2017). Furthermore, obesity rates are higher in Māori, Pacific and European/other women than men, while overweight rates are higher in Māori, Pacific, European/other and Asian men than women.

Childhood obesity rates in New Zealand have also increased over the last few decades. It is reported that 21% of children between the ages of 2–14 years are overweight and a further 12%

obese. After adjusting for age and gender, Māori and Pacific children were more likely to be obese than non-Māori and non-Pacific children respectively (Ministry of Health, 2017).

**Table 2.1 2016/2017 Overweight and obesity rates in New Zealand**

Population	Group	Overweight (%) 25–29.9 kg/m <sup>2</sup>	Obese (%) ≥30 kg/m <sup>2</sup>
Total population	Overall	34.3	32.2
	Men	39.1	30.5
	Women	29.9	33.8
European/other	Overall	36.2	30.5
	Men	41.2	28.6
	Women	31.5	32.2
Māori	Overall	28.3	50.2
	Men	29.5	48.8
	Women	27.2	51.5
Pacific	Overall	20.7	68.7
	Men	24.1	63.6
	Women	17.7	73.4
Asian	Overall	33.8	14.8
	Men	38.6	15.8
	Women	28.5	13.6

Data retrieved from the 2016/17 New Zealand Health Survey (Ministry of Health, 2017).

### 2.3.3 Health and economic consequences of obesity

Obesity has many health, psychological, social and economic consequences for the individuals and for society as a whole. The adverse health consequences of overweight and obesity have been extensively discussed (Guh *et al.*, 2009; Berenson, 2012; Cheng *et al.*, 2016). High BMI and adiposity are associated with increased risk of developing many non-communicable diseases, including type 2 diabetes, hypertension, dyslipidaemia, stroke, ischaemic heart disease, chronic kidney disease and osteoarthritis (DiPietro *et al.*, 1994; Asia Pacific Cohort Studies Collaboration., 2004; Hall *et al.*, 2014; Flego *et al.*, 2016; Brown *et al.*, 2000). In addition, high BMI predisposes individuals to several types of cancers, including uterine, gallbladder, kidney, cervical, thyroid, liver, colon, ovarian and postmenopausal breast cancer (Martin-Rodriguez *et al.*, 2015; Jenabi and Poorolajal, 2015; Bhaskaran *et al.*, 2014).

Obesity related co-morbidities are associated with reduced quality of life, increased sedentary lifestyle and higher levels of disability (Flego *et al.*, 2016). Furthermore, obesity is associated with increased mortality rates. For example, a BMI ≥40 kg/m<sup>2</sup> compared to a normal-weight BMI was associated with elevated mortality rates with most of the deaths caused by heart disease, cancer and type 2 diabetes (Kitahara *et al.*, 2014).

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Over the past decade, stigma relating to body size has received increased recognition. The experience of weight stigma or discrimination is associated with many psychological problems including depression, anxiety and low self-esteem (Rudisill *et al.*, 2016). Furthermore, body size and weight stigma can negatively influence motivation to exercise, thereby resulting in more weight gain (Vartanian and Novak, 2011).

Obesity adds to the increased economic cost, both direct (medical) and indirect (non-medical), imposing a significant economic burden. Direct costs include all direct medical and non-medical costs for diagnosis, treatment and hospitalisation (Lal *et al.*, 2012; Johnson *et al.*, 2016; Vellinga *et al.*, 2008; Withrow and Alter, 2011). In the United States, the direct healthcare costs associated with obesity is estimated to double every decade and by 2030 these costs are expected to range from \$860.7 to \$956.9 billion (Wang *et al.*, 2008). In New Zealand, the cost of lost productivity due to obesity lay between \$98 to \$225 million and the estimated healthcare cost relating to overweight and obesity is 4.4% of the total healthcare expenditure (\$624 million) (Lal *et al.*, 2012). It has been discussed that as a result of the health consequences, obesity indirectly contributes to higher economic costs through reduced productive hours, working at reduced capacity and higher disability benefit payments (Trogon *et al.*, 2008).

Obesity during pregnancy has many short-term and long-term adverse health consequences for the mother and offspring. Maternal obesity is associated with increased risk of gestation diabetes, pre-eclampsia, pre-term birth and large-for-gestational-age babies (Marchi *et al.*, 2015; Catalano and Shankar, 2017; Crane *et al.*, 2009). Furthermore, maternal obesity is linked with increased risk of long-term health consequences to the offspring, such as obesity during childhood, cardiovascular disease (e.g., high blood pressure, adverse lipid profile), insulin resistance, asthma, allergies and behavioural and emotional problems (Catalano and Shankar, 2017; Crane *et al.*, 2009; Marchi *et al.*, 2015; Gaillard, 2015).

Given the many detrimental health, social and economic consequences, there is a significant need to understand factors driving and promoting weight gain and obesity, in particular, to understand factors that increase the risk of obesity in women of reproductive age in order to slow down the progression of obesity.

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### 2.3.4 Causes of obesity

The causes of obesity are both multifactorial and complex (Haslam and James, 2005). Several factors identified as potential contributors of obesity include, diet and physical activity patterns, physiological changes associated with early-life nutrition, genetic factors, influence of gut microbiome and social and economic factors (Albuquerque *et al.*, 2015; Hill *et al.*, 2003).

According to the simplest definition, obesity reflects a state of positive energy balance and arises as a consequence of how the body regulates energy intake, energy expenditure and energy storage (Hall *et al.*, 2011). Although the human body to a certain extent can compensate for changes in energy balance, the compensatory physiological changes cannot maintain body weight in a continuous state of positive energy balance, resulting in excess energy being stored as fat (Horton *et al.*, 1995; Blundell *et al.*, 2003). Physical activity patterns have changed over the last few decades towards being more physically inactive and leading more sedentary lives (Hallal *et al.*, 2012). Furthermore, the modern Western diet consists of highly processed food with excessive amounts of sugar, salt and fat (Swinburn *et al.*, 2011). The modern lifestyle of increased energy intake and lower physical activity are considered significant drivers of the current obesity rates (Swinburn *et al.*, 2011; Hallal *et al.*, 2012; Vandevijvere and Swinburn, 2014). Obesity involves a state of increased insulin secretion, systemic insulin resistance, increased oxidative stress, increased leptin secretion, inflammation and a decreased ability to metabolise lipids, consequently resulting in energy stored as adipose tissue (Saltiel, 2012; Thompson *et al.*, 2007; Hayes and Dinkova-Kostova, 2014; Carlson *et al.*, 2009). In addition, changes in the action of endocrine regulators including insulin, leptin, ghrelin and GLP-1 disturb appetite regulation in the obese state, resulting in sustained weight loss difficult to achieve (Sumithran *et al.*, 2011).

Another causal pathway to obesity has been described by the developmental origins of health and disease (DOHaD) hypothesis. It highlights the link between the periconceptual, foetal and early infant phases of life and the subsequent development of adult obesity and related metabolic disorders (Gluckman and Hanson, 2008). The DOHaD model illustrates that the foetus makes adaptations in response to cues from the intrauterine environment, resulting in permanent adjustments that support the survival and improve success in the postnatal environment (Gluckman and Hanson, 2008). One such adaptation is linked with the exposure to an early life nutritionally limited environment, resulting in an epigenetic process that is more likely to be mismatched in a later energy-rich environment, thus, increasing the risk of metabolic compromise (Kensara *et al.*, 2005). Another adaptation is linked with foetal overnutrition, where

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changes in adipogenesis and/or appetite regulation mechanisms in-utero affect later development in a manner that can also influence obesity (Gluckman and Hanson, 2008).

Several studies have shown that genetic factors play a significant role in obesity development (Farooqi and O'Rahilly, 2006; Xia and Grant, 2013). There are several well-known gene mutations of obesity that code for proteins of the leptin-melanocortin signalling pathway (e.g., leptin receptor, pro-opiomelanocortin), thereby affecting regulation of food intake and energy expenditure (Albuquerque *et al.*, 2015). The genetic profile of polygenic obesity results from the effects of several altered genes, such as gene variants that affect body weight (e.g., melanocortin-4 receptor gene, fat mass and obesity associated gene) (Albuquerque *et al.*, 2015). The mechanisms of how an individual's genetic makeup contribute to obesity is complex and not completely understood, but most often involves the interaction between genes and the environment (i.e., diet, lifestyle factors) (Farooqi and O'Rahilly, 2006).

Other factors identified as potential contributors of increased risk of obesity include poor sleep quality and decline in cigarette smoking (Filozof *et al.*, 2004; Beccuti and Pannain, 2011). Some studies have shown a negative correlation between the hours of sleep per night and BMI, while sleep restriction has also been shown to increase hunger and appetite (Beccuti and Pannain, 2011). The relatively recent decline in cigarette smoking may also be a factor contributing to increased obesity rates, as studies have shown that weight gain is common following smoking cessation (Filozof *et al.*, 2004).

Experimental evidence of the importance of microbiome for metabolism of energy has led to understanding the role of the microbiome in the obesity epidemic (Mathur and Barlow, 2015; Million *et al.*, 2013). One of the important activities of the large intestinal microbiota is to break down substrates such as resistant starch and dietary fibre. The products from this fibre breakdown (e.g., short chain fatty acids) are utilised for lipid or glucose de novo synthesis (Mathur and Barlow, 2015). Two gut microbe categories, Bacteroidetes and Firmicutes, are receiving considerable attention as relative proportions of both differ in obese and non-obese individuals and due to the changes in the composition of the microbiome following weight loss (Ley *et al.*, 2006). However, more research is needed to understand the role of microbiome in obesity. Specifically, to understand the mechanisms by which different profiles of gut microbiota harvest energy from the diet, regulate appetite and glucose and lipid metabolism (Castaner *et al.*, 2018).

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### 2.3.5 The obesogenic food environment

The production of ultra-processed, inexpensive and successfully marketed foods is creating an 'obesogenic' food environment leading to passive over-consumption of sugar- and fat-rich food (Swinburn *et al.*, 2011; Popkin and Gordon-Larsen, 2004; Vandevijvere and Swinburn, 2014). Sugar and fat are concentrated sources of energy with rewarding post-ingestive effects and significant sensory appeal (Drewnowski, 1997b; Stice *et al.*, 2013).

The adverse health effects of excess sugar consumption have been examined for decades with claims that increased intakes are associated with an increased risk of weight gain, obesity and many health conditions, including heart disease, type 2 diabetes, dental caries and fatty liver disease (Te Morenga *et al.*, 2013; Rodríguez *et al.*, 2016; Bray and Popkin, 2014). Inadequate study designs, differences in dietary assessment methods, inconsistent findings between studies and varying definitions of sugars have resulted in some studies showing no evidence of a relationship between sugar intake and the development of obesity or type 2 diabetes (Stanhope, 2016; Kahn and Sievenpiper, 2014). However, the most consistent association has been between high intakes of sugar-sweetened beverages and the development of obesity and metabolic disease (Te Morenga *et al.*, 2013; Narain *et al.*, 2017; Malik *et al.*, 2006).

Literature on the relationship between excess fat intake and obesity is also inconsistent. While some studies show that dietary fat does affect obesity (Bray and Popkin, 1998; Curb and Marcus, 1991), others argue that dietary fat is not important in the development of obesity (Kratz *et al.*, 2013; Willett, 2002). Nevertheless, it is clear that obesity is a state of positive energy balance and that foods high in added sugar and fat are palatable, energy-dense and can lead to higher total calorie consumption (Drewnowski, 2007; Hill, 2006). Thus, highlights the need to understand factors influencing the type and amount of sugar- and fat-rich foods consumed.

#### 2.3.5.1 The global nutrition transition

Analyses of economic and food availability data reveal a major shift in the structure of the global diet in the early 1990's driven by many factors including urbanisation, economic growth and culture (Popkin and Gordon-Larsen, 2004; Popkin, 2006; Drewnowski and Popkin, 1997). These factors have had a major impact on diet with an increased consumption of both sugar and fat observed independent of income (Drewnowski and Popkin, 1997).

Sugar (sucrose) is the world's predominant sweetener (Popkin and Gordon-Larsen, 2004). A large increase in caloric intake of sugars (glucose, fructose, sucrose) and sweeteners (honey, maple syrup, high-fructose corn syrup, dextrose) was observed throughout the world between

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the period of 1960 to 2000 (Popkin and Nielsen, 2003). The caloric intake of sugar (sucrose) increased considerably in low- and middle-income countries than high-income countries over this period (Popkin and Nielsen, 2003; Popkin and Gordon-Larsen, 2004). Furthermore, the characteristic positive association between income level and fat intake observed in the 1960's changed rapidly during the intervening four decades, resulting in higher consumption of fat (animal products and vegetable fat) in low- and middle-income countries (Drewnowski and Popkin, 1997). Additionally, as the world economy grew, differences in the diet between countries with different levels of income were less marked.

The impact of economic factors on the nutrition transition is particularly apparent in Asian countries. Food consumption data from 21 Asian countries between 1975–1994 showed an overall decrease in energy from complex carbohydrates but a corresponding increase in energy from total fats (Drewnowski and Popkin, 1997). The rise in the 'Western style' diet is also observed in many developing countries in Asia, where the leading cause of diet-related non-communicable diseases is linked to the increased total sugar, fat and salt consumption from highly processed foods and beverages (Baker and Friel, 2014; Zhai *et al.*, 2014; Baker and Friel, 2016).

Several dietary factors (i.e., dietary diversity, eating behaviour factors, dietary patterns) play a role in the development of obesity. Over the past few decades food intake has shifted towards increased consumption of highly processed and energy-dense foods in the form of sugar sweetened beverages, sweets and desserts, red and processed meats, ready-made meals and fast-food (Popkin, 2006; Rouhani *et al.*, 2014; Alkerwi *et al.*, 2015), which has resulted in higher energy intake and a change in the macronutrient composition towards higher intakes of sugar and fat.

The positive association between the increase in non-communicable disease and changes in food, nutrition and dietary habits are highlighted in the 2010 Global Burden of Disease report (Lim *et al.*, 2012). Launched in 2012, the International Network for Food and Obesity/non-communicable diseases Research, Monitoring and Action Support (INFORMAS) is a global network of public-interest organisations and researchers that aim to monitor, benchmark and support public and private sector actions to create healthy food environments and reduce obesity and non-communicable diseases (Swinburn *et al.*, 2013). The INFORMAS also support governments, international agencies (e.g., WHO, Food and Agricultural Organisation), private sector and civil society organisations in their efforts to implement policies and actions to improve the healthiness of food environments (Swinburn *et al.*, 2013). Furthermore, the WHO

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Global Action Plan for the Prevention and Control of Non-Communicable Diseases highlights the need to develop policy measures to improve the availability, affordability and acceptability of healthier food products and create healthier food environments with reduced intakes of sugar and fat (World Health Organisation, 2013).

The WHO recommends reducing the intake of free sugars to less than 10% of total energy intake and a further reduction to less than 5% for additional health benefits (World Health Organisation, 2015a). Free sugars include monosaccharides and disaccharides added to foods and beverages and sugars naturally present in honey, syrups, fruit juices and fruit juice concentrates (World Health Organisation, 2015a). The latest New Zealand National Nutrition Survey reports that the median daily intake of total sugars (free sugars and added) was 96 g for females (Ministry of Health, 2011). Furthermore, the median daily intake of carbohydrate was 207g (47%) for females, which falls within the acceptable macronutrient distribution range (AMDR) of 45–65% energy from carbohydrate (National Health and Medical Research Council, 2006). In addition, the intake of free sugar was approximately 10%, in line with the recommendations by the WHO. The most significant contributors of the daily intake of total sugar was sucrose, followed by fructose, glucose and lactose (Ministry of Health, 2011). The main sources of sucrose were sugar and sweets, non-alcoholic beverages, cakes, muffins and biscuits, while the main sources of fructose were fruit, non-alcoholic beverages, vegetables, sugar and sweets and alcoholic beverages (Ministry of Health, 2011). With regards to fat intake, the WHO recommends less than 30% of total energy intake from fats, reducing saturated fats to less than 10% and trans fats to less than 1% of total energy intake and replacing both with unsaturated fats (World Health Organisation, 2015b).

### **2.3.5.2 Influence of sensory attributes on food intake**

Sensory attributes such as taste, smell, mouthfeel and texture directs us towards food sources (e.g., sweet taste indicating nutritious food), guides preferences and portion selection, indicates fullness after food intake and facilitates dietary learning (McCrickerd and Forde, 2016). Of the different sensory attributes, taste perception is one of the most significant contributors of food choice and intake (Honkanen and Frewer, 2009 ; Kourouniotis *et al.*, 2016). Therefore, there has been an increased interest in understanding the importance of food's sensory characteristics on food choice and intake, as sensory cues present at the time of eating can promote over-consumption, especially energy-dense foods rich in added sugar and fat (Cox *et al.*, 1999).

Food palatability is defined as the positive hedonic liking associated with food's sensory characteristics (Yeomans, 1998). It has been shown that increased palatability can promote

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over-consumption as people tend to eat more of the foods they rate as more palatable (Sorensen *et al.*, 2003). Furthermore, it is well known that sugar- and fat-rich foods have high sensory appeal, generate positive food reward responses and that the combination of sugar and fat can induce higher hedonic liking compared to less energy-dense food (Warwick and Schiffman, 1990; Nasser, 2001; Drewnowski, 1997b; Drewnowski, 1995).

Many studies have investigated the association between hedonic liking of sugar- and fat-rich food and obesity (Deglaire *et al.*, 2015; Cox *et al.*, 1999). Hedonic liking is commonly assessed using questionnaires where participants rate on a hedonic scale their liking of the individual food items that are either sweet, fatty or both. A few studies have shown a positive association between sweet and fat hedonic liking and obesity, possibly driven by over-consumption of sugar and fat rich foods (Lampuré *et al.*, 2016; Deglaire *et al.*, 2015; Dressler and Smith, 2013; Duffy *et al.*, 2009). Although individuals generally like (e.g., sweet) and dislike (e.g., bitter) similar tastes, the preferred concentration of sweet and fat tastes varies widely between individuals (Mennella *et al.*, 2011). Furthermore, the same concentration of sweet or fat tastant can be detected and perceived differently between individuals (Low *et al.*, 2016; Tucker and Mattes, 2013). These differences in sweet and fat taste perception could drive food choice and eating habits as people tend to eat more of the foods containing their most preferred concentration. Therefore, the sense of taste is an important contributor of food intake influencing the type and amount of sweet and fatty tasting foods consumed.

## **2.4 Evaluation of taste perception**

Sensory evaluation is a scientific discipline that measures and evaluates responses to certain characteristics of food as they are perceived by sight, smell, taste, touch or hearing (Stone *et al.*, 2012). Three scientific fields, physiology, psychology and psychophysics have contributed to the development of the principles associated with sensory evaluation methods (Moskowitz and Meiselman, 1977). Physiology contributed to information regarding taste receptors and the signal transduction pathways of taste perception, while psychology contributed to the understanding of psychological aspects relating to food product evaluation. During the mid-19<sup>th</sup> century, Weber and then Fechner building on Weber's observations, studied the relationship between the physical and psychological fields giving rise to psychophysics that helped understand the true relationship between stimulus and response (Stone *et al.*, 2012). Information gained from each of these scientific fields has had a major influence on the development of various sensory evaluation methods known as psychophysical measurements (Stone *et al.*, 2012).

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Traditionally, sensory evaluation was used by sensory scientists to assess consumer responses to food and beverage products with the aim of increasing knowledge about consumer preferences and behaviour to maximise sales of food product and profit (Jellinek, 1985). Over the past few decades the use of sensory evaluation approaches has moved beyond food product analysis towards health research, assessing the link between taste and food intake (Drewnowski *et al.*, 1999; Stewart *et al.*, 2010), and taste and obesity (Stewart and Keast, 2012; Salbe *et al.*, 2004; Martinez-Cordero *et al.*, 2015).

There are four main types of psychophysical measurements commonly used to assess taste perception; detection threshold, recognition threshold, taste intensity and hedonic liking (Lawless and Heymann, 1999). Each of these psychophysical measurements characterise a different component of taste perception (Webb *et al.*, 2015).

#### **2.4.1 Detection and recognition thresholds**

Identifying a taste quality first requires the tastants (e.g., sucrose, caffeine) to dissolve in saliva in order to bind to taste receptors in the gustatory system (Frank and Hettinger, 2005). When a tastant is dissolved in a background solution at very low concentrations, a difference over the background solution (e.g., water) is not identifiable. However, as the concentration of the tastant increases, a difference above the background solution is detected but the quality of the tastant is not identified. Detection threshold is defined as the lowest concentration of a tastant detected as different over the background solution (Lawless and Heymann, 1999). As the concentration of the tastant is increased further, a recognition threshold is reached. Recognition threshold is defined as the lowest concentration of a tastant required to successfully recognise the quality of the taste (i.e., sweet or bitter) (Lawless and Heymann, 1999). Detection and recognition thresholds are considered as biological measures of an individual's ability to detect or recognise a taste.

The two commonly used psychophysical methods of threshold determination are the staircase method (Nakamura *et al.*, 2008; Pepino *et al.*, 2012) and the alternative forced-choice ascending method (Leek, 2001; Stewart *et al.*, 2010). In the staircase method, the participant's threshold level is determined using an ascending and descending concentration series. Participants are given one sample containing the tastant and one or more background samples and asked to identify either the sample that is different from the background solution or identify the sample with a recognisable taste. If the participant's choice is incorrect, the subsequent concentration is increased. If the correct sample is identified, the subsequent concentration is decreased (Leek, 2001). This change in the direction of concentration is referred to as a reversal and the

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procedure is called staircase method as the ascending and descending reversals can be connected to look like a staircase (Pasquet *et al.*, 2006; Cornsweet, 1962).

The staircase method has a few advantages in comparison to other approaches. Firstly, this method requires a fewer number of tastant samples compared to other psychophysical tests. Once the first concentration is tested the remaining concentrations are focused closer to the threshold value and each reversal refines the process of determining the threshold measurement (Cornsweet, 1962). Secondly, this method allows re-testing samples previously not identified, thereby giving the participant a chance to improve their accuracy of detection, thus reducing the variability in threshold data (Tucker and Mattes, 2013). One disadvantage of the staircase method is the error of habituation, when participants become accustomed to reporting that they perceive a stimulus and may continue reporting the same way even beyond the threshold. Another disadvantage is the error of anticipation, when the participant anticipates an increase or decrease in the stimulus and may make a premature judgment. Due to these reasons, a modification was introduced to the staircase method. The ascending and descending rules were adjusted to include a certain number of correct or incorrect judgments before changing the direction of the tastant concentration, rather than only one correct or incorrect judgment at one concentration (Kunka *et al.*, 1981).

The second method of threshold determination is the alternative forced-choice ascending method. As the name suggests, this method involves participants evaluating tastant samples from the lowest concentration through to the highest. At each concentration level, a set of samples, one containing the tastant and one or more background samples are presented. The participant is then asked to identify which sample is different from the background sample (detection threshold) or the sample with a recognisable taste (recognition threshold). This procedure is called forced-choice as the participants are asked to select a sample even if they are not able to differentiate between them (Stone *et al.*, 2012; Lawless and Heymann, 1999). Depending on the number of samples presented at a given concentration level, this method is called 2-alternative forced-choice or 3-alternative forced-choice ascending method. If the participant identifies the correct sample, one or more samples of the same concentration are presented and the participant is required to identify the correct sample at every trial. Failing to do so would lead to tasting the higher concentration until three consecutive correct choices are made at one concentration or until all concentration levels are tried (ASTM E679-04, 2004).

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## 2.4.2 Methodological issues of threshold testing

There are many important methodological factors associated with threshold testing that can influence the accuracy of thresholds and introduce variability in the data.

Firstly, tastants can be presented to the participants in many different ways, including tastants soaked in filter paper (Mueller *et al.*, 2003), taste tablets (Ahne *et al.*, 2000) or as aqueous solutions either placed on specific points of the tongue or taste the whole sample at once (Webb *et al.*, 2015). These different sample presentation methods lead to tastants being delivered in a whole mouth or regional manner, leading to variations in the amount of saliva produced, thereby introducing variability. One study reported that the recognition threshold obtained using taste tablets were generally higher compared to placing liquid tastants on the tongue. The difference in threshold values was attributed to taste tablets requiring more saliva to dissolve the tastants and as a result the tastant concentration was lower compared to liquid drops placed on the tongue (Ahne *et al.*, 2000).

Secondly, the number of concentration levels used to assess thresholds can vary between studies ranging from four (Mueller *et al.*, 2003) to eight concentration levels (Ahne *et al.*, 2000). The number of concentration levels determine the amount of time required to complete threshold testing and with more concentration levels fatigue or sensory adaptation may be experienced, causing variability in threshold data (Lawless and Heymann, 1999).

The third methodological factor that can introduce variability is the type of stopping rule, also known as the point where threshold testing is terminated. The stopping rule is dependent on the type of psychophysical method used. For example, in the case of the staircase method, the stopping rule usually is a predetermined number of reversals (Cornsweet, 1962; Pepino *et al.*, 2012). In the ascending forced-choice method, commonly used stopping rules include complete evaluation of all concentration levels or terminating threshold testing when a predetermined number of correct samples are identified (Peng *et al.*, 2012).

The type of stopping rule determines the probability of identifying the correct sample purely by chance (Peng *et al.*, 2012). For the alternative forced-choice method, the probability of identifying the correct sample by chance would depend on the number of samples presented at each concentration level. For example, in the 3-alternative forced-choice method, if a stopping rule of three consecutive correct identifications is applied, then there is a 3.7% probability of identifying the correct sample purely by chance. The probability of identifying the correct sample by chance for less conservative stopping rules such as two correct identifications at one concentration or a single correct identification at one concentration is 11.1% and 33.3%

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respectively (Peng *et al.*, 2012; Lawless, 2010). In theory, the conservative stopping rules would significantly reduce identifying the correct sample purely by chance. In practice, a conservative stopping rule requires more time to complete the task and may introduce participant fatigue, adaptation or reduced motivation to complete the task, all influencing the precision of threshold measurements (Lawless and Heymann, 1999).

Another methodological factor to consider when determining taste thresholds is the way in which the best-estimate threshold also known as the final threshold value is calculated for each participant. The allocation of the best-estimate threshold is determined by the stopping rule used. For example, the best-estimate threshold for the staircase method is the geometric mean of the lowest concentration of each reversal (Pasquet *et al.*, 2006) or the mean concentration of the last four reversals (Nakamura *et al.*, 2008). For the ascending forced-choice method, the most common best-estimate threshold is the concentration at which three consecutive correct identifications were made. If the participant selects the incorrect sample at the highest concentration, then the geometric mean of the highest concentration and the next theoretical higher concentration is often calculated (ASTM E679-04, 2004).

It is clear that many important methodological details contribute to within- and between-subject and between-studies variation in threshold measurements. Furthermore, differences in methodological details make it difficult to compare methods and findings between studies. This emphasises the need for evaluating and validating threshold testing protocols within each experimental setting. Optimising the methodological aspects will eliminate the variability introduced by the methodology so the true biological variability of taste thresholds can be confidently measured.

### **2.4.3 Taste intensity and hedonic liking**

Tastant concentrations above thresholds produce a perception of taste intensity, which is defined as the magnitude of sensation evoked by different tastant concentrations (Keast and Breslin, 2002). Furthermore, concentrations above thresholds are used to evaluate hedonic liking, an indicator of an individual's liking for a particular tastant concentration (Lim, 2011). Tastant concentrations used to measure taste intensity and hedonic liking are known as suprathreshold concentrations, as they are above threshold level where the quality of the taste is known (e.g., sweet, salty) (Lawless and Heymann, 1999). The measurement of taste intensity and hedonic liking is essential to evaluate relationships between taste and nutrition-related health outcomes as suprathreshold concentrations relate better to the tastant concentrations experienced in real-world foods and beverages (Duffy *et al.*, 2009; Bartoshuk *et al.*, 2004a). Taste

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intensity and hedonic liking cannot be measured directly as we cannot share each other's sensory experiences. Therefore, indirect methods such as rating the perceived taste intensity and hedonic liking of one or more suprathreshold concentrations is used to quantify these perceptions (Bartoshuk *et al.*, 2003; Stone *et al.*, 2012). Three main types of rating scales have been used in past research.

The oldest and most widely used rating method is the category scale, which consists of verbal descriptors of varying levels of perceived taste intensity or hedonic liking (Stone *et al.*, 2012). The 9-point scale is an example of a category scale commonly used in research which can be used for taste intensity measurements by anchoring the bottom end as 'not at all bitter' and top end as 'extremely bitter' or for hedonic liking measurements by anchoring the bottom end as 'dislike extremely' and top end as 'like extremely' (Figure 2.5) (Kaminski *et al.*, 2000).

Overall, how much do you like or dislike this juice sample?

Sample 351

<input type="checkbox"/>	Like extremely
<input type="checkbox"/>	Like very much
<input type="checkbox"/>	Like moderately
<input type="checkbox"/>	Like slightly
<input type="checkbox"/>	Neither like nor dislike
<input type="checkbox"/>	Dislike slightly
<input type="checkbox"/>	Dislike moderately
<input type="checkbox"/>	Dislike very much
<input type="checkbox"/>	Dislike extremely

**Figure 2.5 The 9-point hedonic scale**

Reprinted from Food Quality and Preference. Lim, J., 2011, Hedonic scaling: A review of methods and theory, 2011, with permission from Elsevier.

One of the main advantages of the category scale is the ease of use. However, a few issues limit the use of this type of scale. Firstly, due to the lack of a reference point, taste perception comparisons between individuals cannot be derived easily (Bartoshuk *et al.*, 2006). Secondly, this scale introduces ceiling effects where the participant's responses gather around the limited categories. Thirdly, it is not known whether the descriptors are equally spaced giving the scale a ratio property for comparing taste perceptions between groups (Bartoshuk *et al.*, 2003). These issues led to the development of a second method, namely, a line marking scale which involves the participant making an indication along a line to mark the strength of the perceived taste intensity or hedonic liking (e.g., 100 mm VAS) (Lawless and Heymann, 1999). In this method, the

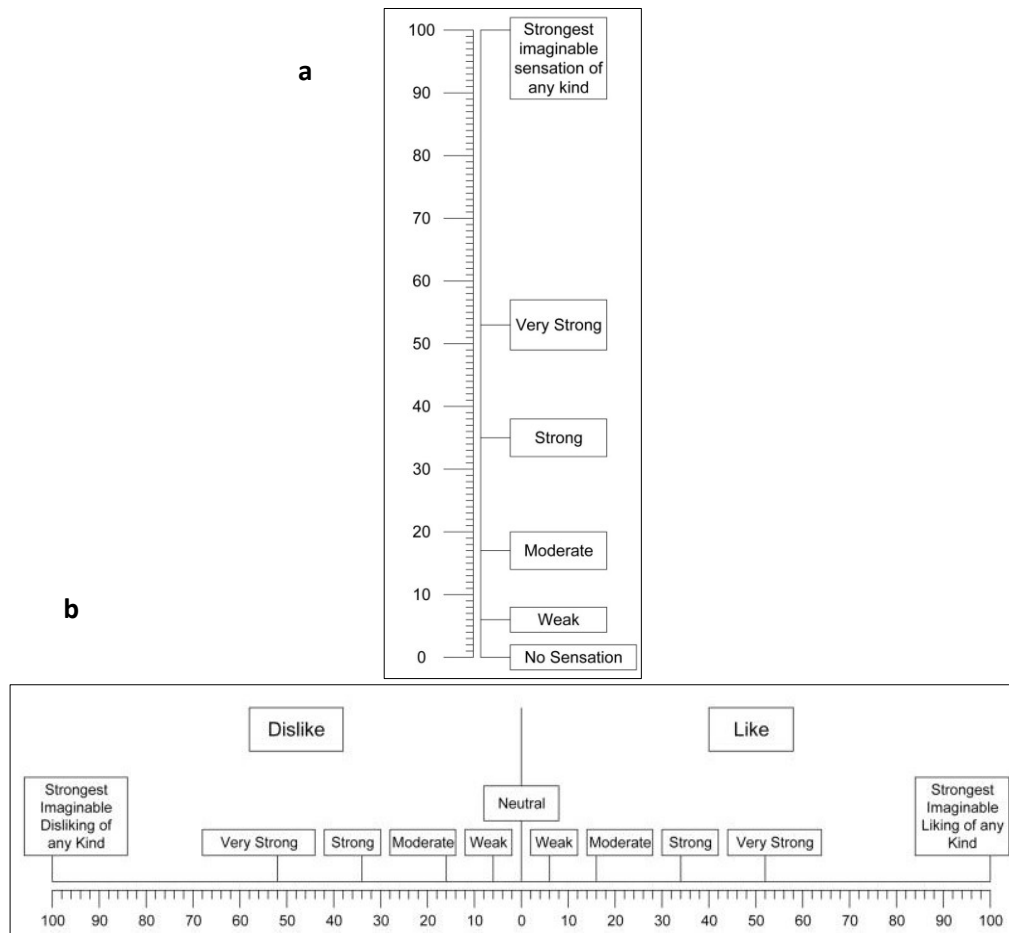
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two ends of the scale are anchored with words denoting extreme perceptions and participants can mark their response anywhere along the line giving the VAS a ratio property (Bartoshuk *et al.*, 2003).

Interpreting taste intensity and hedonic liking within an individual is straightforward. The comparison of sensory experiences between different individuals or groups is more complex as these experiences cannot be shared directly (Bartoshuk *et al.*, 2006). In the mid-1900s S.S. Stevens introduced a direct scaling method known as the method of magnitude estimation, which involved a sensation twice as intense to be assigned a number that was twice as large (Stevens, 1969; Bartoshuk *et al.*, 2003). However, as Stevens was interested in comparing different sensory modalities and not between-subject comparisons, he did not evaluate if magnitude estimation is valid for group comparisons (Stevens, 1969).

Adjective labelled scales were then used for within-subject and between-group comparisons if the participants in each group were assigned randomly (Bartoshuk *et al.*, 2003). However, this method was not suitable to measure taste intensity or hedonic liking differences between groups that differ in age, ethnic group or weight, as the same verbal descriptor may mean different experiences or the same line marking on a scale may be produced by different perceptions (Bartoshuk *et al.*, 2006). These reasons lead to the development of the labelled magnitude scale by Green and colleagues (Green *et al.*, 1993).

The labelled magnitude scale is a semantic scale of intensity, characterised by a quasi-logarithmic spacing of its descriptors such that it is larger in space when moved from weak to strong perceptions. The labelled magnitude scale has been shown to yield psychophysical functions equivalent to the gold standard method of magnitude estimation (Green *et al.*, 1996). A further improvement to this scale was made by Bartoshuk and colleagues, who anchored the top of the labelled magnitude scale with 'strongest imaginable sensation of any kind' (Bartoshuk *et al.*, 2004b). This scale was then referred as the gLMS and could be used for taste or any other perception measurements. The gLMS is a labelled scale with ratio properties and allows valid across group comparisons (Bartoshuk *et al.*, 2003). The gLMS can be used to rate taste intensity (Figure 2.6a) or hedonic liking (Figure 2.6b) and the rating will be done in reference to the 'strongest imaginable sensation of any kind' experienced by each individual (Bartoshuk *et al.*, 2004a).



**Figure 2.6 The general labelled magnitude scale**

The general labelled magnitude scale used for rating taste intensity (a) and hedonic liking (b). Reprinted from *Annals of the New York Academy of Sciences*. Cruickshanks *et al.*, 2009, Measuring taste impairment in epidemiologic studies. The Beaver Dam Offspring Study, 2009, with permission from John Wiley and Sons.

## 2.5 Sweet and fat taste perception and dietary intake

The basic biology of accepting nutrient-rich sweet and salty tastes and rejecting bitter and sour tastes is evident from an early age where new-borns show differential facial responses to these tastes (Rosenstein and Oster, 1988). Children’s taste perception is usually linked with liking sweet taste, disliking bitter taste and eating more regularly the food they like (Drewnowski *et al.*, 2012; Mennella *et al.*, 2011). However, the link between sweet and fat taste perception and dietary intake in adults is not straightforward. Higher intakes of sweet and fatty foods are not always linked with higher preferences for these tastes but are influenced by many economic, behavioural and cultural factors (Koster, 2009; Drewnowski and Darmon, 2005). Taste is a significant driver of dietary choice and intake (Kourouniotis *et al.*, 2016; Wandel and Bugge, 1997) and it is therefore important to understand the link between different characteristics of sweet and fat taste perception and dietary intake.

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### 2.5.1 The influence of early-life sensory experiences on dietary intake

There are three critical windows of early-life sensory experiences (i.e., exposure to amniotic fluid, milk feeding and introduction to solid foods) that can shape taste preferences in childhood and influence food preferences carried through to adulthood (Mennella, 2014).

A child's first experience of taste takes place when the foetus swallows amniotic fluid from the second trimester onwards (Sase *et al.*, 2005). It has been reported that the mother's diet during pregnancy can influence the composition of the amniotic fluid and may influence subsequent acceptance of food by the infant (Mennella *et al.*, 1995; Schaal *et al.*, 2000). For example, Mennella *et al.*, 2001 reported that infants born to mothers who consumed carrot juice for 3 weeks during the last trimester exhibited less negative facial responses to carrot-flavoured cereal compared to infants born to mothers who consumed water. Schaal *et al.*, 2000 reported that infants born to mothers who consumed anise during pregnancy had a preference for the flavour. Furthermore, amniotic fluid of mothers with gestation diabetes has elevated glucose concentrations. This leads to foetal macrosomia due to the increased glucose that passes through the placenta into the foetal circulation and is stored as body fat (Kc *et al.*, 2015; Tisi *et al.*, 2011). Overall, these findings suggest that the composition of the amniotic fluid can influence early chemosensory learning and may guide early-life taste preferences of the offspring. We could speculate that the exposure to a high sweet environment at such an early stage in life may predispose the offspring towards liking higher concentrations of sweet taste, higher intake of sugar and adverse metabolic health consequences long-term.

During the early postnatal period, breast milk or formula is the first medium of flavour learning (Mennella, 1995). The association between the mother's diet during breastfeeding and the acceptance of certain types of food by the infant during the weaning stage has been demonstrated by a few studies. Mennella *et al.*, 2001 reported that infants born to mothers who consumed carrot juice during the first two months of breastfeeding showed fewer negative facial responses to carrot-flavoured cereal compared to infants whose mothers consumed water. Another study conducted by Mennella *et al.*, 2009 reported that the type of formula fed to infants had an effect on their taste response. For example, infants on hydrolysed casein formula that has a pronounced bitter, sour and savoury taste, consumed significantly more bitter, sour and savoury cereals than infants who consumed breast milk or bovine milk based formula (Mennella *et al.*, 2009). These studies support the notion that flavour learning during the milk feeding period may guide an infant's taste perception towards foods that have flavours experienced during this stage.

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During the first year of an infant's life there is a transition from milk based foods to a diet consisting of complementary solid foods. Children have a higher preference for sweet and fat tastes due to evolutionary driven preferences that programme children to like tastes that are energy-dense (Mennella *et al.*, 2014; Kern *et al.*, 1993). Early-life taste experiences could influence a child's diet, especially in the contemporary setting where children have access to many readily-available foods and beverages high in added sugar and fat (Swinburn *et al.*, 2011; Monteiro *et al.*, 2013). It has been shown that increase acceptance of healthy foods can be achieved by repeated exposure and a more diversified diet can be achieved by introducing a variety of healthy foods with different tastes and textures (Mennella and Trabulsi, 2012; Forestell and Mennella, 2007; Myers *et al.*, 2005; Maier *et al.*, 2007). Therefore, the nature of the foods introduced during the solid food period may be critical for future taste acceptances (Harris and Coulthard, 2016). More importantly, this period of sensory learning provides an opportunity to introduce a variety of healthy foods that may promote a healthier start to later-life diet (Forestell and Mennella, 2007; Harris and Coulthard, 2016).

Overall, the critical windows of early-life taste experiences provide an opportunity to intervene at an early stage to change childhood taste preferences. In particular, to reduce the exposure to high concentrations of sweet and fat tastes and offer healthier foods in order to reduce the predisposition to obesity through reduced intakes of highly process, highly palatable, energy-dense sweet and fatty foods (Forestell and Mennella, 2007; Harris and Coulthard, 2016).

### **2.5.2 Assessment of dietary intake**

Unhealthy diet and eating habits are major lifestyle-related risk factors for a wide range of chronic illnesses (Hays *et al.*, 2002; Bray and Popkin, 1998). Capturing information on diet is difficult due to within- and between-subject variations in food intake introduced by both biological and environmental influences such as appetite, seasonal differences in food intake and economic factors (Shahar *et al.*, 2001). Dietary intake assessment is a comprehensive evaluation of food intake, which includes information on the quality of diet, consumption of nutrients and food groups and dietary patterns (Thompson and Subar, 2013). In sensory research, many studies have assessed the link between sweet and fat taste perception and food intake using dietary intake data obtained from three main methods; 24-hour diet recall, food record and FFQ (Low *et al.*, 2016; Mattes, 1985; Chevrot *et al.*, 2014). Table 2.2 provides a brief description, strengths and limitations of these dietary assessment methods.

**Table 2.2 Dietary assessment methods commonly used in sensory research**

Method	Description of method	Strengths	Limitations
24-hour diet recall	<ul style="list-style-type: none"> <li>Retrospective method</li> <li>Interviewer asks the participants to recall all foods and beverages consumed over the last 24 hours, including quantity, brands and preparation methods</li> </ul>	<ul style="list-style-type: none"> <li>Low participant burden as administration time is short</li> <li>Can be conducted either in person or over the phone</li> <li>Higher reliability due to personal contact</li> <li>No literacy requirements needed as administered by interviewer</li> </ul>	<ul style="list-style-type: none"> <li>Requires a skilled interviewer to identify nutritional habits, types of foods and preparation methods</li> <li>Relies on participant's memory</li> <li>Often difficult to estimate portion sizes</li> <li>Multiple 24 hour recalls necessary to determine usual intake</li> <li>Potential interviewer bias</li> <li>A single recall not representative of usual intake, but can be used to characterise average intakes</li> </ul>
Food records (weighed or estimated)	<ul style="list-style-type: none"> <li>Prospective method</li> <li>Participant records all foods and beverages consumed over a period at the time of consumption</li> <li>Weighed food record – food consumed is weighed with scales</li> <li>Estimated food records – household measures and food models to estimate portion sizes</li> <li>Measures actual current dietary intakes</li> </ul>	<ul style="list-style-type: none"> <li>Weighed food records considered the 'gold standard' method of determining energy and macronutrient intakes</li> <li>Less reliance on memory</li> <li>Accurate measure of food intake as all details about food consumption including quantity, preparation methods, brands and time of consumption are recorded</li> </ul>	<ul style="list-style-type: none"> <li>Participants need to be motivated and literate</li> <li>High participant burden</li> <li>Requires training prior to recording to improve recording accuracy</li> <li>Habitual dietary patterns can be altered during the course of recording due to participant fatigue</li> <li>If information on intake are not recorded at the time of consumption, a number of foods may be omitted</li> </ul>
Food frequency questionnaire	<ul style="list-style-type: none"> <li>Retrospective method</li> <li>Participants report usual frequency of consumption of a list of foods over a certain period (month/s or year)</li> <li>Can be quantitative if portion sizes are included or qualitative if only frequency of intakes are measured</li> <li>Usually administered as a questionnaire</li> <li>Measures usual dietary intakes</li> </ul>	<ul style="list-style-type: none"> <li>Low burden on participants</li> <li>Easy to administer and low cost</li> <li>Less impacted by dietary changes as it captures dietary intake over a period of time</li> <li>Can be used in large study populations</li> </ul>	<ul style="list-style-type: none"> <li>Relies on memory</li> <li>Over- and under-reporting of intake</li> <li>Relies upon self-reporting and ability of participant to make generic quantifications of recent intakes</li> <li>Doesn't usually assess preparation methods or combinations of foods consumed together</li> </ul>

The above table was assembled using references Biro *et al.*, 2002 and Gibson, 2005.

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One of the major challenges of nutrition research is obtaining accurate records of food intake (Thompson and Subar, 2013). It is important that the dietary assessment method is reliable and valid to ensure the dietary information of the study population is determined accurately (Vucic *et al.*, 2009). Self-reported dietary data often suffer from measurement error, known as the difference between the measured value and the true value (Subar *et al.*, 2015). Furthermore, across all dietary assessment methods the most well documented challenge is misreporting of energy intake (Subar *et al.*, 2015; Gemming *et al.*, 2014). Several factors associated with misreporting of dietary data include age, gender, weight status, physical activity and cognitive factors (Gemming *et al.*, 2014; Maurer *et al.*, 2006). It has been suggested that studies should collect dietary data from both short-term (diet recalls or food records) and long-term (FFQs) methods to allow for maximising the strengths of each method. Furthermore, dietary data should be interpreted whilst keeping in mind the limitations of the dietary assessment method (Subar *et al.*, 2015).

### **2.5.3 Cross-sectional studies assessing the link between sweet taste perception and dietary intake**

Although humans have an innate preference for sweet taste (Rosenstein and Oster, 1988), inter-individual variations of how sweet taste is detected, perceived and liked can influence food preference, choice and intake (Low *et al.*, 2016; Asao *et al.*, 2015). Table 2.3 reports the outcomes of the cross-sectional studies that have investigated the link between sweet taste perception and dietary intake in adults. It can be hypothesised that individuals with a lower sweet detection or recognition threshold (i.e., more sensitive) would be able to detect the sweetness of food at a lower concentration or would require lesser amounts of sweet food to be satisfied with the taste. Most of the studies generally agree that the lowest concentration of sweet taste that is detected or recognised is not correlated with total energy or intakes of carbohydrate, starch or sugar (Table 2.3) (Low *et al.*, 2016; Mattes, 1985; Martinez-Cordero *et al.*, 2015). It is generally thought that taste thresholds relate poorly with real-world eating experiences as thresholds only reflect the lower end of tastant concentrations, possibly explaining the lack of relationship between sweet taste thresholds and dietary intake (Bartoshuk *et al.*, 2006).

On the other hand, taste intensity of suprathreshold concentrations is considered a more appropriate measure to assess links between taste and dietary intake (Low *et al.*, 2016). Most studies found no robust relationships between sweet taste intensity and intakes of total energy, carbohydrate and total sugar, frequency of sweet food consumption or eating behaviours

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relating to sweet food consumption (Table 2.3) (Mattes, 1985; Holt *et al.*, 2000; Mahar and Duizer, 2007; Cicerale *et al.*, 2012). In contrast, Low *et al.*, 2016 found a positive correlation between total energy intake and sweet taste intensity ratings for Rebaudioside A (stevia) and sucralose ( $r=0.4$ ,  $p<0.01$ , Table 2.3). This study suggests that the magnitude of sweet sensation experienced is weakly associated with energy intake.

As explained in Table 2.3, several studies to date have found a link between hedonic liking of sweet taste and dietary measurements. For example, a few studies have reported a positive correlation between the preferred level of sucrose and the frequency of sweet food and refined sugar intakes (Holt *et al.*, 2000), preference for sweet desserts and the amount of sugar in tea (Drewnowski *et al.*, 1999) and the sugar content of favourite cereal (Mennella *et al.*, 2011). In addition, individuals who consumed higher amounts of sweetened beverages preferred sweeter sucrose solutions than those who consumed lesser amounts of sweetened beverages (Table 2.3) (Mahar and Duizer, 2007). These findings generally suggest a weak positive association between sweet hedonic liking and dietary measurements.

**Table 2.3 Cross-sectional studies investigating the relationship between sweet taste perception and dietary intake in adults**

Reference & country	Participants	Dietary measurements	Psychophysical measurements of sweet taste	Main outcomes of the study
(Mattes, 1985) United States	17 males & 18 females, 18–42 years	7-day diet record (estimated)	Recognition thresholds of sucrose solutions and food systems (cherry flavoured beverage) using the forced-choice staircase method	No association between recognition thresholds (sucrose solution or food system) and percent energy from carbohydrate, fat, protein or sugar
			Perceived sweet intensity of five sucrose samples (0.05–0.8 M) using the magnitude matching procedure	No association between perceived intensity and percent energy from carbohydrate, fat, protein or sugar
			Preferred sucrose concentration determined by allowing the participants to dilute and concentrate sucrose samples	Preferred sweet concentration was not related to percent energy from carbohydrate, fat, protein or sugar
(Mattes and Mela, 1986) United States	25 males, 17–34 years	7-day diet record (estimated), food frequency survey of 30 sweet foods	Adjustment task to determine the preferred sweetness level of five oatmeal samples	Positive correlation between preferred sucrose concentration in oatmeal and percent energy from sweet foods (carbohydrate and sugar) ( $r=0.49-0.62$ , $p<0.01$ )
			Adjustment task to determine the preferred sweetness level of coffee	Positive correlation between preferred sucrose concentration in coffee and percent energy from sugar ( $r=0.49$ , $p<0.01$ ) and frequency of sweet food selection ( $r=0.54$ , $p<0.01$ )
(Drewnowski <i>et al.</i> , 1999) United States	159 females, 20–60 years	Food preference questionnaire, 3-day food record (estimated)	Rate the sweet hedonic liking of five sucrose samples (2, 4, 8, 16 & 32%) on a 9-point hedonic scale	Positive relationship between the mean hedonic liking of all sucrose samples and liking ratings of sweet desserts and sugar in tea ( $r=0.2-0.3$ , $p<0.05$ )
(Holt <i>et al.</i> , 2000) Australia	56 males & 76 females, 17–35 years	FFQ to assess habitual intake of sugar, artificial sweeteners, sweet foods and sweet drinks	Rate the perceived sweet intensity of sucrose samples, orange juice, custard samples and shortbread cookies with varying levels of sucrose	No relationship between perceived sweet intensity and frequency of sweet food consumption or intake of refined and total sugar
			Rate the sweet hedonic liking of sucrose samples, orange juice, custard samples and shortbread cookies with varying levels of sucrose	Positive correlations between the sum of the preferred level of sucrose across all four taste stimuli and frequency of consumption of sweet foods and intake of refined sugars ( $r=0.3$ , $p<0.05$ )

Reference & country	Participants	Dietary measurements	Psychophysical measurements of sweet taste	Main outcomes of the study
(Mahar and Duizer, 2007) Canada	64 females, 18–54 years	Questionnaire assessing the frequency of sweetened beverage intake and type of sweetener predominantly consumed (artificial vs natural)	Rating the perceived intensity of five orange juice samples with varying levels of sucrose (0, 5, 10, 15 & 20%) on a 9-point category scale Rating the hedonic liking of five orange juice samples with varying levels of sucrose (0, 5, 10, 15 & 20%) on a 9-point category scale	No differences in sweet intensity rating of orange juice between sweetener group (artificial vs natural) or sweetened beverage intake groups (high vs low intake) No significant differences in sweet hedonic liking ratings of orange juice between sweetener group High sweetened beverage intake group had higher sweet hedonic liking ratings compared to the low beverage intake group ( $p<0.05$ )
(Mennella <i>et al.</i> , 2011) United States	356 children (5–10 years), 169 adolescents (10–20 years), 424 adults (20–55 years)	Sugar content of favourite cereal	Preferred sucrose levels using the Monell 2-series forced-choice sucrose preference test	Sucrose preference correlated positively with the sugar content of their favourite cereal ( $r=0.11$ , $p<0.001$ )
(Cicerale <i>et al.</i> , 2012) Australia	9 males & 76 females, 17–25 years	Dietary activities and food beliefs questionnaire, food variety survey, 2 * 24-hour food records	Rating the perceived intensity of 200 mM sucrose on a gLMS	No relationship between perceived intensity and food variety score, total energy intake, percent energy from fat, protein, carbohydrate or food behaviours relating to sugar consumption
(Martinez-Cordero <i>et al.</i> , 2015) Mexico	30 males & 26 females, 24–43 years	7-day food record (estimated)	Recognition threshold of sucrose and aspartame using the 2-alternative forced-choice ascending method	No relationship between sucrose threshold and energy intake Aspartame threshold negatively correlated with energy intake (multiple regression $\beta=-0.003$ , $p<0.001$ )

Reference & country	Participants	Dietary measurements	Psychophysical measurements of sweet taste	Main outcomes of the study
(Low <i>et al.</i> , 2016) Australia	28 males & 32 females, 18–52 years	FFQ	Detection and recognition thresholds of glucose, fructose, sucrose, sucralose, erythritol and Rebaudioside A using the 3-alternative forced-choice method  Rating the perceived sweetness of glucose, fructose, sucrose, sucralose, erythritol and Rebaudioside A on a gLMS	No correlations between sweet thresholds and total energy intake or percent energy from total fat, protein, carbohydrate, sugar, starch or fibre  Only a positive correlation between total energy intake and sweetness intensity ratings for Rebaudioside A ( $r=0.4$ , $p<0.01$ ) and sucralose ( $r=0.36$ , $p<0.01$ )

FFQ: food frequency questionnaire, gLMS: general labelled magnitude scale. Age of participants reported as range.

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#### **2.5.4 Intervention studies assessing the link between sweet taste perception and dietary intake**

A few studies have reported a change in sweet taste perception following dietary interventions. A study by Wise *et al.*, 2015 investigated whether a 3-month dietary intervention of 40% less calories from simple sugars (low-sugar group) would influence sweet taste perception compared to a control group who maintained their usual food intake. This study found no change in sucrose thresholds or sweet pleasantness ratings in response to the dietary intervention. However at the third month, the low-sugar group rated both low and high concentrations of sucrose as sweeter than the control group, indicating an increased sweet sensitivity in response to the low-sugar diet (Wise *et al.*, 2015).

Sartor *et al.*, 2011 investigated the effects of a one month excess energy intake of 600 kcal/day in the form of a soft drink supplementation on sweet taste perception in a group of 12 normal-weight individuals. This study found that the soft drink supplementation altered sweet taste intensity such that a weak sucrose concentration (10 mM) was perceived as sweeter and the stronger concentration (178 mM) was perceived as less sweet compared to the intensity perceived before the intervention. In addition, following the soft drink supplementation a decrease in pleasantness was observed only at 100 mM sucrose compared to the pre-intervention ratings (Sartor *et al.*, 2011).

A recent study by Noel *et al.*, 2017 investigated the effects of diminished sweet taste intensity (treatment with varying concentrations of tea containing *Gymnema sylvestre*) on hedonic liking of sweet solutions and sweet foods. This study found that diminished sweet intensity (i.e., reduced sensitivity) was linked with an increased desire for foods containing higher amounts of sucrose. This suggests that lower sweet taste intensity can shift sweet hedonic liking towards foods with higher sucrose content to attain a satisfactory level of reward (Noel *et al.*, 2017).

Overall, the intervention studies discussed above show that sweet taste intensity and hedonic liking can be altered by dietary manipulations. However, these studies did not assess whether altered sweet taste perception lasts beyond the duration of the intervention or translates to changes in actual consumption of sweet tasting foods.

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### 2.5.5 Cross-sectional studies assessing the link between fat taste perception and dietary intake

Since the discovery of putative receptors of fatty acid detection within the gustatory system, many studies have investigated the link between fat taste perception and dietary intake. Table 2.4 summarises the main findings of the cross-sectional studies investigating the relationship between different measures of fat taste perception and dietary intake in adults. Several studies found differences in food intake between individuals with different fatty acid detection abilities. For example, some studies showed that individuals who were hypersensitive to oleic acid (i.e., more sensitive) consumed less total energy, fat and carbohydrate compared to those who were hyposensitive (Stewart *et al.*, 2010; Stewart *et al.*, 2011a). A recent study by Costanzo *et al.*, 2017 reported that oleic acid detection thresholds correlated positively with percent energy from fat, negatively with percent energy from carbohydrate and positively with the frequency of high-fat dairy, meat, meat alternatives, grains and cereal consumption (Table 2.4). These findings suggest that lower fat taste sensitivity is related with higher intakes of energy and certain macronutrients and higher frequency of high-fat food intake. In contrast, Keast *et al.*, 2014 found no differences in energy or macronutrient intakes between oleic acid hypersensitive and hyposensitive individuals (Table 2.4).

Rating fat taste intensity and ranking samples according to fat content have been used as markers of fat taste sensitivity when investigating the link between fat taste perception and dietary intake. Martínez-Ruiz *et al.*, 2014 reported that those who rated fat taste as more intense had a lower preference for high-fat food, consumed Mexican street food less frequently and consumed smaller amounts of fast-food compared to those who found the samples less intense (Table 2.4). Furthermore, a study conducted by Liang *et al.*, 2012 reported that individuals who were unable to discriminate between different fat concentrations consumed more added fats such as butter, spreads, oils and low-fat food compared to fat discriminators (Table 2.4). These findings suggest that a lower perceived fat taste intensity is related with higher intakes of fatty foods.

The relationship between fat hedonic liking and food intake has been explored in only a handful of studies. A recent study reported that individuals with a higher hedonic liking for ice cream with higher amounts of fat consumed more dairy products compared to those who liked ice cream with lower amounts of fat (Table 2.4) (Shen *et al.*, 2017). However, there is general agreement that no relationship exists between hedonic liking of fat taste and total energy or fat intake (Mela and Sacchetti, 1991; Shen *et al.*, 2017).

**Table 2.4 Cross-sectional studies investigating the relationship between fat taste perception and dietary intake in adults**

Reference & country	Participants	Dietary measurements	Psychophysical measurements of fat taste	Main outcomes of the study
(Mela and Sacchetti, 1991) United States	9 males & 21 females, 27.5±7 (SD) years	10-day food record (estimated)	The mean preferred level of fat across 10 different foods each prepared with 2–5 levels of fat	No correlation between the most preferred level of fat and percent energy from fat
(Stewart et al., 2010) Australia	6 males & 48 females, 20.0±3.6 (SEM) years	2-day food record (weighed)	Participants screened for detecting 1.4 mM oleic acid and grouped as hypersensitive ( <i>n</i> =12) or hyposensitive ( <i>n</i> =42) Fat ranking test using custard containing varying amounts of oil (0, 2, 6 & 10%)	Hypersensitive participants consumed less energy and absolute amounts (g) of fat and carbohydrate compared with hyposensitive participants ( <i>p</i> <0.05) Hypersensitive participants had better ability to rank custards based on fat content than hyposensitive participants ( <i>p</i> <0.05)
(Stewart et al., 2011a) Australia	10 males & 41 females, 20.8±0.5 (SEM) years	4-day food record, food attitudes and behaviour questionnaire	Participants screened for detecting 3.8 mM oleic acid and grouped as hypersensitive ( <i>n</i> =13) and hyposensitive ( <i>n</i> =30) Fat ranking test using custard containing varying amounts of oil (0, 2, 6 & 10%)	Hyposensitive participants consumed significantly more energy and absolute amounts (g) of fat, saturated fat and polyunsaturated fat than hypersensitive participants ( <i>p</i> <0.05) Hyposensitive participants had higher intakes of full-fat dairy, saturated fat from dairy, meat, eggs and spreads ( <i>p</i> <0.05) Hypersensitive participants had better ability to rank custards based on fat content than hyposensitive participants ( <i>p</i> <0.05)
(Liang et al., 2012) United States	137 males & 180 females, 18–65 years	Food frequency and preference questionnaire	Participants were given seven Italian salad dressing samples varying in fat content (5–55%) to screen for fat discriminators ( <i>n</i> =59) and fat non-discriminators ( <i>n</i> =33)	Fat non-discriminators reported higher intakes of both added fats (butters, fat spreads, oils) and reduced and low-fat foods (fat-free and low-fat dairy products and snacks) compared to fat discriminators ( <i>p</i> <0.05)
(Keast et al., 2014) Australia	14 males, 24.0±8.4 (SD) years & 10 females, 32.0±14.3 (SD) years	4-day food record	Participants screened for detecting 3.8 mM oleic acid and grouped as hypersensitive ( <i>n</i> =14) and hyposensitive ( <i>n</i> =10)	There was no significant difference in energy or macronutrient intakes between hypersensitive and hyposensitive participants

Reference & country	Participants	Dietary measurements	Psychophysical measurements of fat taste	Main outcomes of the study
(Chevrot et al., 2014) France	59 males, 52.3±2.1 (SEM) years	2*24-h food records	Linoleic acid detection threshold to identify linoleic acid taster groups; Lean tasters ( $n=25$ ), lean non-tasters ( $n=4$ ), obese tasters ( $n=25$ ) and obese non-tasters ( $n=5$ )	Obese non-tasters consumed more energy ( $p<0.0007$ ), carbohydrates ( $p<0.0003$ ), fat ( $p<0.0079$ ) and saturated fat ( $p<0.01$ ) compared to obese tasters
(Martínez-Ruiz et al., 2014) Mexico	43 males & 78 females, 21.1±3.6 (SD) years	Food record questionnaire, food preference questionnaire	Rated the fat taste intensity of four linoleic acid concentrations and grouped into four quartiles; low (QL), medium–low (QML), medium–high (QMH) and high (QH)	Energy and macronutrient intakes were not significantly different between the intensity ratings groups  The QH group reported lower preference for high-fat foods (e.g., fast-food and Mexican street foods) ( $p<0.01$ ), consumed Mexican street foods less frequently ( $p=0.04$ ), and consumed smaller amounts of fast-food ( $p=0.04$ ) and Mexican street foods ( $p=0.03$ ) than QL group
(Shen et al., 2017) United Kingdom	41 males & 95 females, 18–55 years	FFQ, 3-day food record	Rate the hedonic liking of ice cream samples containing varying amounts of fat on a 9-point hedonic category scale to create liking clusters; non-discriminators (like ice cream regardless of fat), high-fat disliker (like low-fat ice cream) and high-fat liker (like high-fat ice cream)	Liking clusters had no significant relationship with total energy or fat intake  High-fat liker group had higher dairy intake compared to high-fat dislikers ( $p=0.052$ )
(Costanzo et al., 2017) Australia	69 females, 18–62 years	24-h diet recall, food liking questionnaire, FFQ	Oleic acid detection threshold using the 3-alternative forced-choice ascending method	Oleic acid detection threshold correlated positively with percent energy from fat ( $\beta=0.11$ ) and negatively with percent energy from carbohydrate ( $\beta=-0.11$ )  Oleic acid detection threshold correlated positively with the frequency of consumption of high-fat dairy ( $\beta=0.11$ ), meat & meat alternatives ( $\beta=0.67$ ) and grain & cereals ( $\beta=0.77$ )

SD: standard deviation, SEM: standard error of the mean, FFQ: food frequency questionnaire, gLMS: general labelled magnitude scale.

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### **2.5.6 Intervention studies assessing the link between fat taste perception and dietary intake**

A few intervention studies have shown links between fat taste perception and diet. Stewart and Keast, 2012 evaluated the change in oleic acid threshold in response to a four-week dietary intervention of a high-fat (45% energy from fat) or low-fat (15% energy from fat) diet. In response to the high-fat diet, an increase in fatty acid thresholds (i.e., reduced sensitivity) was evident in lean individuals but not in obese individuals. On the other hand, the low-fat energy diet decreased fatty acid thresholds (i.e., increased sensitivity) in lean and obese individuals (Stewart and Keast, 2012). A study by Newman *et al.*, 2016 assessed the effects of a 6-week low-fat (25% fat) or portion control (33% fat) diet on fat taste perception in overweight/obese individuals. Both diets lead to a decrease in fat taste thresholds (i.e., more sensitive), but the effect tended to be stronger in the low-fat diet group compared to the portion control diet group. Furthermore, the ability to perceive different fat concentrations in foods was increased after the low-fat diet intervention (Newman *et al.*, 2016). These studies suggest that dietary interventions can modify fat taste perception. If changes in taste perception in response to dietary interventions translate to changes in food choice and intake is yet to be explored.

### **2.5.7 Reasons for inconsistent findings between studies**

Several methodological factors associated with sensory testing and dietary assessment methods may explain the discrepancies in findings between sweet and fat taste perception and dietary intake. With regards to taste perception measurements, several factors associated with sensory testing can introduce variability and cause inconsistencies between studies. For example, the vehicle solution (e.g., water or milk), the type of tastant (e.g., sucrose, glucose, oleic acid, canola oil) and details associated with sensory testing (e.g., concentration range and levels, sample presentation method, type of rating scales) differ between studies. These differences can produce vastly different taste perceptions, making the comparison of methods and results between studies difficult. Furthermore, sweet and fat tastants presented as aqueous solutions (e.g., sucrose dissolved in distilled water) may not emulate real-world eating experiences (e.g., sugar-sweetened beverages) and may influence taste intensity and hedonic liking measurements. Real-food systems (e.g., varying concentrations of sucrose in beverages, popcorn with varying levels of fat) may better depict foods and beverages consumed in real-life. Therefore, a combination of pure tastant samples and real-food systems may relate better with dietary intake measurements. It is still unclear which measure of taste perception (i.e., thresholds, taste intensity or hedonic liking) is better correlated with dietary intake as each

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measurement characterises a different component of taste (Webb *et al.*, 2015). Therefore, a combination of taste perception measurements may provide a more comprehensive assessment of taste function.

Dietary measurements used in different studies may be inadequate to assess actual intakes, thereby not capturing accurate intakes of energy, macronutrients and sugars. Although a few studies have used food records (mostly estimated), most studies have used FFQs to obtain energy and macronutrient intakes. As discussed, the weighed food record is the gold standard method for nutrient intake assessment as participants maintain records of their actual dietary intake at the time of consumption (Biro *et al.*, 2002). Other types of questionnaires assessing food preference, habitual intakes of sugar and sweet beverages, dietary activity and food beliefs may not capture the information required to robustly assess the link between sweet and fat taste perception and dietary intake. Furthermore, if the dietary intake range of the study participants is too narrow or not sufficiently varied, relationships between dietary intake and taste perception will be difficult to ascertain. This may further explain why some studies have found relationships between sweet and fat taste perception and dietary intake while others have not.

There are many variables associated with the study design that can contribute to the inconsistent findings. Most studies that have assessed the link between taste and dietary intake are cross-sectional in nature rather than intervention studies. This may provide insight into some of the discrepancies in findings as differences in taste perception following a dietary intervention may be more significant. Furthermore, it is known that with increasing age, sensitivity to taste decreases while hedonic liking for high concentrations of sweet also decrease (Mennella *et al.*, 2011; Mojet *et al.*, 2003). Most studies that have assessed the relationship between taste perception and food intake recruited participants with a large age range (Costanzo *et al.*, 2017; Liang *et al.*, 2012) and age-related differences in taste perception may be masked. Furthermore, most studies have small sample sizes which may not provide enough dietary variation to identify relationships between taste perception and dietary intake. Therefore, future studies should assess these relationships in larger cohorts, with a narrow age range and valid and appropriate taste perception and dietary measurements to determine the true link between sweet and fat taste perception and dietary intake.

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## 2.6 Sweet and fat taste perception and body composition

Over-consumption of sugar and fat is linked with excess energy intake, thus a significant contributor of weight gain (Bray *et al.*, 2004; Malik *et al.*, 2006; Te Morenga *et al.*, 2013; Bray and Popkin, 1998). A lower taste sensitivity and/or a higher hedonic liking for sweet and fat tastes could lead to over-consumption of sugar- and fat-rich foods. Therefore, it is of interest to understand whether an individual's perception of sweet and fat taste is a factor contributing to weight gain and obesity. Thus, this part of the review examines the association between different measurements of sweet and fat taste perception and body composition.

### 2.6.1 Cross-sectional studies assessing the link between sweet taste perception and body composition

In 1958, Pangborn & Simone concluded that sweet food liking was not systematically different across body sizes. Furthermore, some of the earliest work in this area failed to find any links between body weight and sweet taste perception, as neither thresholds or suprathreshold measures were found to be different across body weight groups (Frijters and Rasmussen-Conrad, 1982; Malcolm *et al.*, 1980; Thompson *et al.*, 1976; Pangborn and Simone, 1958). Since the earlier work in this area, the psychophysical tools with which we compare sensory experiences across groups have improved. Many recent studies have investigated differences in sweet taste perception measurements between BMI groups as new techniques may detect differences that the old techniques missed (e.g., use of valid rating scales).

Table 2.5 summarises the findings of the recent cross-sectional studies investigating the relationship between sweet taste perception and body composition in adults. A few studies have explored whether sweet taste thresholds differ between BMI groups. While the majority of studies found no significant link between sweet taste thresholds and BMI (Pepino *et al.*, 2010; Skrandies and Zschieschang, 2015; Park *et al.*, 2015; Martinez-Cordero *et al.*, 2015; Low *et al.*, 2016; Simchen *et al.*, 2006), a few others report conflicting results. For example, two studies reported that overweight individuals have higher detection thresholds compared to normal-weight individuals (Table 2.5) (Ettinger *et al.*, 2012; Proserpio *et al.*, 2016). In contrast, Hardikar *et al.*, 2017 found that obese individuals have lower recognition thresholds compared to lean individuals. Furthermore, a recent study by Fernandez-Garcia *et al.*, 2017 found that morbidly obese individuals (BMI >40 kg/m<sup>2</sup>) had a lower ability to recognise sweet taste compared to normal-weight individuals (Table 2.5).

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As discussed in Table 2.5, with regards to the link between sweet taste intensity and BMI, while some studies report no associations (Low *et al.*, 2016; Salbe *et al.*, 2004; Pepino *et al.*, 2010), others report that overweight/obese individuals find sucrose samples less intense (Sartor *et al.*, 2011; Ettinger *et al.*, 2012) or more intense than do lean individuals (Hardikar *et al.*, 2017). A few studies have assessed the link between sweet taste hedonic liking and obesity. With the exception of Hardikar *et al.*, 2017 showing that obese individuals find high concentrations of sucrose more pleasant than do lean individuals, no robust link between sweet hedonic liking and obesity has been reported (Table 2.5) (Sartor *et al.*, 2011; Ettinger *et al.*, 2012; Pepino *et al.*, 2010).

**Table 2.5 Cross-sectional studies investigating the relationship between sweet taste perception and body composition in adults**

Reference & country	Participants	Body composition measurements/groups	Psychophysical measurements of sweet taste	Main outcomes of the study
(Salbe <i>et al.</i> , 2004) United States	123 Pima Indians & 64 Caucasians, 32.7±10 (SD) years	BMI, body fat %	Rate the sweet intensity of four sucrose samples (0, 5, 10 & 20%) on a 100 mm VAS Rate the pleasantness of four sucrose samples (0, 5, 10 & 20%) on a 100 mm VAS	BMI or body fat % was not a significant predictor of sweet intensity BMI or body fat % was not a significant predictor of sweet pleasantness
(Simchen <i>et al.</i> , 2006) Germany	130 males & 181 females, 20–88 years	BMI groups of ≥28 kg/m <sup>2</sup> & <28 kg/m <sup>2</sup>	Recognise the taste quality of four sucrose samples (3.2, 10, 32 & 100 mM)	Recognition of sweet taste was not different between BMI groups
(Pepino <i>et al.</i> , 2010) United States	57 females, 21–40 years	34 normal-weight (<25 kg/m <sup>2</sup> ) & 23 obese (≥29.9 kg/m <sup>2</sup> )	Sucrose detection threshold using the 2-alternative forced-choice staircase procedure Rate the sweet intensity of four sucrose samples (0, 0.09, 0.36 & 1.05 mol/L) on a gLMS Determine the most preferred sucrose solution (0.09, 0.18, 0.35, 0.70 & 1.05 mol/L) using the paired solution tests	Sucrose detection threshold was not different between BMI groups No significant difference in perceived sweet intensity between BMI groups The most preferred sweet concentration was not different between BMI groups
(Sartor <i>et al.</i> , 2011) United Kingdom	14 males & 19 females, 22.8±2.5 (SD) years	22 normal weight (>18–≤25 kg/m <sup>2</sup> ) & 11 overweight/obese (>25 kg/m <sup>2</sup> )	Rate the sweet intensity of 11 sucrose samples (0–1 M) on a gLMS Rate the pleasantness of 11 sucrose samples (0–1 M) on a gLMS	The overweight/obese group perceived all sucrose samples as less intense compared to the normal-weight group ( <i>p</i> <0.05) No differences in pleasantness scores between BMI groups
(Ettinger <i>et al.</i> , 2012) Canada	72 females, 18–49 years	50 normal-weight (18.5–24.9 kg/m <sup>2</sup> ) & 21 overweight (≥25 kg/m <sup>2</sup> ) 47 normal body fat (<26%) & 20 overweight body fat (>26%)	Sucrose detection threshold using the ascending forced-choice method Rate the perceived sweet intensity of four custard samples with sucrose (5, 10, 15 & 20%) on a nine-point category scale	Overweight BMI and body fat groups had significantly higher sucrose thresholds compared to the normal BMI and body fat groups ( <i>p</i> <0.05) Overweight BMI and body fat groups perceived all custard samples as less sweet compared to the normal BMI and body fat groups ( <i>p</i> <0.001)

Reference & country	Participants	Body composition measurements/groups	Psychophysical measurements of sweet taste	Main outcomes of the study
(Ettinger et al., 2012) Canada	72 females, 18–49 years	50 normal-weight (18.5–24.9 kg/m <sup>2</sup> ) & 21 overweight (≥25 kg/m <sup>2</sup> )  47 normal body fat (<26%) & 20 overweight body fat (>26%)	Rate the sweet liking of four custard samples with sucrose (5, 10, 15 & 20%) on a nine-point hedonic scale	No significant difference in sweetness liking between BMI or body fat % groups
(Skrandies and Zschieschang, 2015) Germany	25 males & 41 females, 19–56 years	Four BMI groups; 15–19.9 kg/m <sup>2</sup> (n=11), 20–24.9 kg/m <sup>2</sup> (n=30), 25–29.9 kg/m <sup>2</sup> (n=18) & ≥30 kg/m <sup>2</sup> (n=7)	Saccharose recognition thresholds using four concentrations (0.4, 0.2, 0.1 & 0.05 g/ml)	Saccharose recognition threshold was not significantly different between BMI groups
(Park et al., 2015) South Korea	24 males & 17 females, 20–29 years	23 normal-weight (<23 kg/m <sup>2</sup> ) & 18 obese (>25 kg/m <sup>2</sup> )	Recognising the sweet taste quality of 11 sucrose solutions (0.05–2 g/ml)	Sweet recognition was not different between normal-weight and obese group
(Martinez-Cordero et al., 2015) Mexico	30 males & 26 females, 24–43 years	BMI	Recognition threshold of sucrose & aspartame using the 2-alternative forced-choice ascending method	No significant associations between sucrose or aspartame recognition thresholds and BMI
(Proserpio et al., 2016) Italy	48 males & 55 females, 40.2±10.8 (SD) years	52 normal-weight & 51 obese	Detection threshold of sucrose using the 3-alternative forced-choice method	Obese participants had higher threshold values compared to normal-weight participants
(Low et al., 2016) Australia	28 males & 32 females, 18–52 years	BMI, waist circumference	Detection & recognition thresholds of glucose, fructose, sucrose, sucralose, erythritol and Rebaudioside A using the 3-alternate forced-choice method  Rate the perceived intensity of three concentrations of glucose, fructose, sucrose, sucralose, erythritol and Rebaudioside A on a hedonic gLMS	No correlations between detection or recognition threshold of any sweetener and BMI or waist circumference  No correlations between perceived intensity of any sweetener and BMI or waist circumference

Reference & country	Participants	Body composition measurements/groups	Psychophysical measurements of sweet taste	Main outcomes of the study
(Hardikar et al., 2017) Germany	28 males & 26 females, 18–35 years	31 lean (<25 kg/m <sup>2</sup> ) & 23 obese (>30 kg/m <sup>2</sup> )	Sucrose recognition threshold using an adaptive staircase procedure  Rate the sweet intensity of four sucrose samples (absolute high, absolute low, relative high, relative low) on a visual analogue scale  Rate the pleasantness of four sucrose samples (absolute high, absolute low, relative high, relative low) on a visual analogue scale	Obese participants had significantly lower sweet thresholds than lean participants ( $p=0.01$ )  Obese participants rated the sweet intensity of 'absolute low' and 'absolute high' samples as more intense than lean ( $p<0.05$ )  Obese participants rated the 'relative high' sample as more pleasant than the lean ( $p=0.017$ )
(Fernandez-Garcia et al., 2017) Spain	179 females, 18–65 years	17 low-weight (<18.5 kg/m <sup>2</sup> ), 77 normal-weight (18.5–24.9 kg/m <sup>2</sup> ), 12 overweight (25–29.9 kg/m <sup>2</sup> ), 28 obese (30–39.9 kg/m <sup>2</sup> ), 45 morbid obese ( $\geq 40$ kg/m <sup>2</sup> )	Identify the sweet taste of sucrose samples (0.05, 0.1, 0.2, 0.4 g/ml) impregnated in filter papers	Normal-weight participants had higher sweet recognition scores compared to morbid obese participants

SD: standard deviation, BMI: body mass index, VAS: visual analogue scale, gLMS: general labelled magnitude scale.

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## 2.6.2 Cross-sectional studies assessing the link between fat taste perception and body composition

Investigating the link between fat taste perception and obesity has gained interest in the past decade due to the positive association between higher fat intake and adverse metabolic health consequences (Liu *et al.*, 2016). Table 2.6 summarises the main findings of the cross-sectional studies investigating the link between fat taste perception and body composition in adults.

A few studies have explored links between fatty acid detection thresholds and BMI. Two studies found that individuals that were hyposensitive (i.e., less sensitive) to the detection of oleic acid had significantly higher BMI compared to hypersensitive individuals (Table 2.6) (Stewart *et al.*, 2011a; Stewart *et al.*, 2010). It is important to note that although a difference in BMI was reported between the hyposensitive and hypersensitive groups, the mean BMI of both groups were within the normal-weight range. Proserpio *et al.*, 2016 reported that obese individuals had higher oleic acid detection thresholds (i.e., less sensitive) compared to normal-weight individuals. In contrast to these findings, Chevrot *et al.*, 2014 reported no difference in linoleic acid fatty acid threshold between lean and obese individuals (Table 2.6).

Many studies have shown that BMI groups do not differ in their ability to perceive fat taste intensity (Table 2.6) (Pepino and Mennella, 2014; Salbe *et al.*, 2004; Proserpio *et al.*, 2017). In contrast, Martínez-Ruiz *et al.*, 2014 showed that individuals who perceived linoleic acid samples as more intense had lower BMI and WC than those who perceived the samples as less intense. With regards to fat taste hedonic liking and obesity, while some studies show a positive relationship (Mela and Sacchetti, 1991; Proserpio *et al.*, 2017), others show no associations (Table 2.6) (Salbe *et al.*, 2004; Pepino and Mennella, 2014).

**Table 2.6 Cross-sectional studies investigating the relationship between fat taste perception and body composition**

Reference & country	Participants	Body composition measurements/groups	Psychophysical measurements of fat taste	Main outcomes of the study
(Mela and Sacchetti, 1991) United States	9 males & 21 females, 27.5±7 (SD) years	BMI, body fat	10 different foods, each prepared with 2–5 levels of fat. The mean preferred level of fat across all foods was used as an indicator of overall fat preference.	Total body fat, percent body fat and BMI positively correlated with the most preferred level of fat across all stimuli ( $r=0.46$ , $p=0.005$ )
(Salbe <i>et al.</i> , 2004) United States	123 Pima Indians, & 64 Caucasians, 32.7±10 (SD) years	BMI, body fat %	Rate the creaminess of four milk samples with varying levels of fat (0, 3.5, 11.3 & 37.5%) on a 100 mm visual analogue scale  Rate the hedonic liking of creaminess of four milk samples with varying levels of fat (0, 3.5, 11.3 & 37.5%) on a 100 mm visual analogue scale	BMI or body fat % was not a significant predictor of creaminess ratings  BMI or body fat % was not a predictor of creaminess hedonic liking
(Stewart <i>et al.</i> , 2010) Australia	6 males & 48 females, 20±3.6 (SEM) years	BMI	Participants were divided into two groups; hypersensitive ( $n=12$ ) & hyposensitive ( $n=42$ ) to 1.4 mM oleic acid	BMI was significantly lower in hypersensitive participants (females: 20.2 kg/m <sup>2</sup> and males: 22.6 kg/m <sup>2</sup> ) compared with hyposensitive participants (females: 21.6 kg/m <sup>2</sup> and males: 23 kg/m <sup>2</sup> , $p<0.047$ )
(Stewart <i>et al.</i> , 2011b) Australia	19 males, 19–58 years	8 lean (21.3–25 kg/m <sup>2</sup> ) & 11 overweight /obese (25.9–36.2 kg/m <sup>2</sup> )	Oleic acid detection thresholds determined using the 3-alternative forced-choice method	Detection threshold was significantly higher in overweight/obese (7.9 mmol/L) than in lean participants (4.19 mmol/L, $p<0.05$ )
(Stewart <i>et al.</i> , 2011a) Australia	10 males & 41 females, 18–46 years	BMI	Participants were divided into two groups; hyposensitive & hypersensitive to 3.8 mM oleic acid	BMI was significantly lower in hypersensitive participants (female: 20.4 kg/m <sup>2</sup> and male: 22.3 kg/m <sup>2</sup> ) compared with hyposensitive participants (female: 21.8 kg/m <sup>2</sup> and male: 23.9 kg/m <sup>2</sup> , $p<0.05$ )
(Stewart and Keast, 2012) Australia	31 participants, 35.6±14 (SD) years	19 lean (<24.9 kg/m <sup>2</sup> ) & 12 overweight/obese (>25 kg/m <sup>2</sup> )	Oleic acid detection thresholds determined using the 3-alternative forced-choice method	No significant difference in oleic acid detection threshold between lean and overweight/obese groups

Reference & country	Participants	Body composition measurements/groups	Psychophysical measurements of fat taste	Main outcomes of the study
(Chevrot <i>et al.</i> , 2014) France	59 males, 52.3±2.1 (SEM) years	30 lean (19–25 kg/m <sup>2</sup> ) & 29 obese ≥30 kg/m <sup>2</sup>	Linoleic acid detection threshold using the 3-alternative forced-choice procedure	Linoleic acid detection threshold not different between the lean and obese groups
(Pepino and Mennella, 2014) United States	47 females, 21–41 years	12 normal-weight (18.5–24.9 kg/m <sup>2</sup> ) & 11 obese (>29.9 kg/m <sup>2</sup> )	Rate the perceived creaminess intensity of vanilla puddings with varying fat content (0, 3.1, 6.9 & 15.6%) on a gLMS Rate the pleasantness of vanilla puddings that varied in fat content (0, 3.1, 6.9 & 15.6%) on a hedonic gLMS	No significant difference in perceived creaminess between normal-weight and obese groups No significant difference in pleasantness ratings between normal-weight and obese groups
(Martínez-Ruiz <i>et al.</i> , 2014) Mexico	43 males & 78 females, 21.1±3.6 (SD) years	BMI, waist circumference	According to the fatty acid intensity ratings of four linoleic acid concentrations participants were grouped into four quartiles; low, medium–low, medium–high & high	Participants in the high intensity group had lower BMI values than participants in the low intensity group ( $p=0.04$ ) Waist circumference of participants in the high intensity group (74.7 cm) was significantly lower than participants in the intensity low group (82.1 cm, $p=0.03$ )
(Proserpio <i>et al.</i> , 2016) Italy	48 males & 55 females, 40.2±10.8 (SD) years	52 normal-weight & 51 obese	Oleic acid detection threshold using the 3-alternative forced-choice method	Obese participants had higher threshold values compared to normal-weight participants
(Proserpio <i>et al.</i> , 2017) Italy	44 males & 47 females, 44.8±9.7 (SD) years	45 normal-weight (22.0±2.1 kg/m <sup>2</sup> ) & 46 obese (37.5±5.1 kg/m <sup>2</sup> )	Rate the creaminess of 3 custard samples with varying levels of butter aroma on a gLMS Rate the hedonic liking of 3 custard samples with varying levels of butter on a labelled hedonic scale	No significant differences in creaminess ratings between groups Obese participants had higher liking scores for fat compared to normal-weight participants

SD: standard deviation, BMI: body mass index, SEM: standard error of the mean, gLMS: general labelled magnitude scale.

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### **2.6.3 Longitudinal and intervention studies assessing the link between sweet and fat taste perception and body composition**

Several longitudinal and intervention studies have shown links between sweet hedonic liking and body weight. A study by Salbe *et al.*, 2004 found a positive correlation between the hedonic liking of sweet taste measured at baseline and weight gain after 5 years in a group of obesity prone Pima Indians. Although, participant's hedonic liking was not measured at the follow-up period, this study suggests that heightened hedonic liking may predispose individuals towards weight gain, possibly influenced by higher intakes of sweet foods. Burgess *et al.*, 2016 investigated whether a six-month weight loss intervention in women would influence perceived intensity and hedonic liking of sweet taste. Overall, weight loss did not alter sweet intensity ratings but sweet hedonic liking ratings changed during the trial and indicated a shift towards liking samples with a lower sucrose content compared to samples liked at baseline (Burgess *et al.*, 2016). This suggests that an individual's sweet taste hedonic liking can be altered in response to weight loss. However, well-controlled trials are needed to further establish these findings.

### **2.6.4 Reasons for inconsistent findings between studies**

The inconsistent findings between sweet and fat taste perception and body composition may be attributed to a range of methodological factors associated with sensory testing and the experimental design of the studies. Firstly, factors associated with the psychophysical measurements used in different studies may introduce variations in measurements which can mask true associations. For example, the different types of sweet tastants (e.g., sucrose, aspartame, glucose) and fat tastants (e.g., oleic acid, milk samples), type of rating scales (e.g., gLMS, VAS) and different concentration levels can cause variability in the data, which makes comparing results between studies difficult. Secondly, although BMI is the most commonly used marker of obesity (possibly due to the ease of use), it is not the best predictor of fat mass, especially in different ethnic groups (Romero-Corral *et al.*, 2008; Chiu *et al.*, 2011). The direct assessment of adiposity is an important measure of metabolic health as many adverse health consequences are linked with excess adipose tissue (Henry and Clarke, 2008). Thirdly, differences in participant characteristics (e.g., age, ethnic group, BMI range) may contribute to the inconsistent findings between studies. Fourthly, many studies have relatively small sample sizes and narrow BMI ranges, which may not provide enough statistical power to identify true biological relationships.

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## 2.7 Summary of the literature review

Humans are born hard-wired to like tastes that signal energy-rich nutrients (e.g., sweet taste), indicating a biological drive towards the consumption of energy-dense foods (Rosenstein and Oster, 1988). Many recent studies have shown that early-life taste experiences can serve as a critical window which shape the developing taste preferences that are carried through to adulthood (Ventura and Worobey, 2013; Mennella and Trabulsi, 2012). There are two main factors that predispose children to consume diets high in sugar and fat. One factor is the evolutionary driven innate taste preferences (i.e., likes and dislikes) that are magnified during childhood (Ventura and Mennella, 2011). Another factor is the learned taste preferences through repeated exposure to sugar- and fat-rich foods (Ventura and Mennella, 2011).

The gustatory system is responsible for recognising the five basic taste qualities and more recently fat taste has been added to the nutrient sensing system (Mattes, 2009; Chalé-Rush *et al.*, 2007; Chandrashekar *et al.*, 2006). Traditionally, sensory evaluation was used by sensory scientists to assess consumer responses to food and beverage products with the aim of increasing knowledge about consumer preferences and behaviour to maximise sales of food products (Jellinek, 1985). Over the past few decades the use of sensory evaluation approaches has moved towards health research, assessing the link between taste, dietary intake and obesity (Drewnowski *et al.*, 1999; Stewart *et al.*, 2010; Salbe *et al.*, 2004; Martinez-Cordero *et al.*, 2015).

Taste perception is commonly characterised using four psychophysical measurements; detection threshold, recognition threshold, perceived taste intensity and hedonic liking (Lawless and Heymann, 1999). One of the major challenges of sensory research is accurately measuring the different characteristics of taste perception as many experimental and methodological factors influence taste measurements. For example, type of tastant, concentration levels, type of psychophysical measure, type of rating scale, sample size and characteristics of participants (e.g., age) are known to influence taste measurements (Bartoshuk *et al.*, 2004a; Tucker and Mattes, 2013). Although the above psychophysical measurements are commonly used in research, the relationships between these critical taste measurements have not been rigorously assessed (Webb *et al.*, 2015; Low *et al.*, 2016; Salbe *et al.*, 2004). Therefore, there is a need to critically evaluate the relationships between different psychophysical measurements of taste in order to understand the biological relevance of these measurements and how these measurements can be used to characterise people with different taste sensitivities and preferences (Objective 1 addressed in Chapter 3).

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The alarming increase in obesity rates worldwide has become a major public health crisis as efforts to curb the epidemic have been unsuccessful (The GBD Obesity Collaborators, 2017). Although many causes of obesity have been identified, the changes in the global food system where energy intake exceeds energy needs is considered an important contributor of obesity (Vandevijvere and Swinburn, 2014). The current ‘obesogenic’ food environment consists of easily accessible, inexpensive, highly processed, effectively marketed, nutrient-poor foods that are energy-dense and high in sugar and fat (Vandevijvere and Swinburn, 2014; Swinburn *et al.*, 2011). Consequently, high intakes of the palatable obesogenic diets promote passive over-consumption of energy (Andrieu *et al.*, 2005; Rao *et al.*, 2013; Stubbs and Whybrow, 2004; Mendoza *et al.*, 2007). As passive over-consumption of energy is a significant contributor of weight gain and obesity (Hill, 2006), it is important to understand causes and drivers of dietary choice and intake. Furthermore, sugar and fat are two key ingredients that increase the palatability of foods (Drewnowski and Greenwood, 1983; Drewnowski and Schwartz, 1990). Therefore, understanding the role sweet and fat taste perception plays in dietary choice and intake would further our understanding of factors that drive higher intakes of energy-dense sugar- and fat-rich foods.

Many studies have investigated the link between sweet and fat taste perception and obesity, as differences in taste perception between different BMI groups may influence the type and amount of sweet and fatty foods consumed. To date, there is little agreement whether a relationship between sweet taste perception and obesity exists. Some studies report that overweight/obese individuals have higher sucrose detection thresholds and find sucrose solutions to be less intense compared to normal-weight individuals (Sartor *et al.*, 2011; Ettinger *et al.*, 2012). In contrast, another study found that obese individuals have lower sweet recognition thresholds and perceive sweet solutions as more intense compared to normal-weight individuals (Hardikar *et al.*, 2017). The findings of the studies assessing the link between fat taste perception and obesity are also inconclusive. For example, some studies have shown that individuals who are less sensitive to the detection of fatty acids had higher BMI and WC than who are more sensitive (Stewart *et al.*, 2011a; Stewart *et al.*, 2010; Martínez-Ruiz *et al.*, 2014). However, most studies found no clear link between any sweet or fat taste perception measurement and obesity (Chevrot *et al.*, 2014; Stewart and Keast, 2012; Salbe *et al.*, 2004; Pepino and Mennella, 2014; Low *et al.*, 2016; Park *et al.*, 2015).

Many methodological factors associated with taste perception measurements and the experimental design of studies can be attributed to the discrepancies in findings between the studies. Many studies have used inappropriate rating scales (e.g., VAS) to compare taste

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perception between different study groups (e.g., BMI groups), when the gLMS provides the most robust and only valid comparison (Bartoshuk *et al.*, 2005; Bartoshuk *et al.*, 2004a). Furthermore, factors associated with the experimental design, such as small sample sizes, narrow BMI range and BMI (not body fat) used as the most common measure of obesity are also reasons for the inconsistent findings (Stewart *et al.*, 2011b; Stewart and Keast, 2012; Pepino and Mennella, 2014). Therefore, further investigation in larger cohorts, with well-defined BMI and body fat groups, using appropriate and valid taste perception measurements are needed to address this important knowledge gap in understanding the link between sweet and fat taste perception, obesity and metabolic health (Objective 2 addressed in Chapter 4).

Similar to the prevalence worldwide, overweight and obesity rates in New Zealand adults have increased significantly over the last few decades (Ministry of Health, 2017). There was a significant rise in obesity rates in women of reproductive age between the period of 2006 to 2017. Furthermore, obesity rates are higher in Māori, Pacific and European women than men (Ministry of Health, 2017). Obesity during pregnancy has many short-term and long-term adverse health consequences for the mother and the offspring (Marchi *et al.*, 2015; Catalano and Shankar, 2017; Crane *et al.*, 2009). Therefore, there is a significant need to slow down and reverse the progression of obesity by understanding dietary factors that increase the risk of obesity in women and in ethnic groups who are at higher risk of obesity and metabolic disease.

Dietary pattern analysis has emerged as a complementary method of characterising dietary intakes (Appel *et al.*, 1997; Newby *et al.*, 2004; Hu, 2002). Dietary patterns represent a broader picture of the overall diet as it reflects the combinations of foods and nutrients consumed together. Therefore, dietary pattern analysis is considered a useful tool to assess links between diet and nutrition-related health outcomes (Hu, 2002 ; Moeller *et al.*, 2007). Although many studies have investigated the associations between dietary patterns and health outcomes, to our knowledge dietary patterns of New Zealand populations have only been explored by a handful of studies (Beck *et al.*, 2017; Wall *et al.*, 2016; Thompson *et al.*, 2010). Furthermore, there is a dearth of NZ studies that have explored the links between specific dietary patterns, obesity and metabolic health biomarkers. Therefore, investigating the link between dietary patterns and metabolic health in women from ethnic groups with known differences in metabolic disease and obesity risk is an important knowledge gap that needs to be addressed (Objective 3 addressed in Chapter 5).

A number of studies have investigated the link between different psychophysical measurements of sweet and fat taste perception and dietary intake. With regards to sweet taste perception,

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the overall findings suggest a weak positive correlation between hedonic liking of sweet solutions and some dietary parameters such as the frequency of sweet food and refined sugar intake (Holt *et al.*, 2000) and preferences for sweet desserts and sugar in tea (Drewnowski *et al.*, 1999). However, no robust associations have been found between dietary intake and sweet taste thresholds or sweet taste intensity (Low *et al.*, 2016; Cicerale *et al.*, 2012; Martinez-Cordero *et al.*, 2015). With regards to fat taste perception, some studies have shown a link between lower fat taste sensitivity (i.e., higher detection thresholds and lower perceived intensity) and higher intakes of energy and fat, higher frequency of high-fat food intake and higher preference for high-fat food (Stewart *et al.*, 2010; Stewart *et al.*, 2011a; Costanzo *et al.*, 2017; Martínez-Ruiz *et al.*, 2014). However, there are other studies that have found no significant links between different measurements of fat taste perception and dietary intake (Keast *et al.*, 2014; Shen *et al.*, 2017; Mela and Sacchetti, 1991). Overall, the findings regarding the link between sweet and fat taste perception and dietary intake are not conclusive and highlight an important knowledge gap (Low *et al.*, 2016; Stewart *et al.*, 2011a; Cicerale *et al.*, 2012; Martinez-Cordero *et al.*, 2015).

Several factors associated with taste perception measurements and dietary tools have been identified as potential causes for the inconsistent findings. For example, threshold measurements are often used to assess taste perception when it is suggested that suprathreshold measurements (i.e., perceived taste intensity and hedonic liking) relate better with dietary intake (Bartoshuk *et al.*, 2005; Bartoshuk *et al.*, 2004a). With regards to the dietary tools, most studies use various questionnaires (e.g., FFQ) to measure energy and macronutrient intakes rather than using weighed food records which is the gold-standard method for the assessment of actual dietary intake (Low *et al.*, 2016; Holt *et al.*, 2000; Martínez-Ruiz *et al.*, 2014). There is a dearth of studies that have systematically assessed the relationships between different psychophysical measurements of sweet taste and dietary intake, particularly actual dietary intake assessed using valid dietary tools such as weighed food records. These knowledge gaps highlight the need for further research to evaluate relationships between different psychophysical measurements of sweet taste and various dietary parameters measured using valid sensory and dietary tools (Objective 1 addressed in Chapter 3). Furthermore, no study has assessed the relationship between different patterns of sweet and fat taste perception and dietary intake. In particular, the link with dietary patterns has not been investigated (Objective 4 addressed in Chapter 6). As dietary patterns are easier for the public to interpret and translate into diets when providing dietary guidelines and recommendations, examining the relationships

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between sweet taste and fat (creaminess) perception and dietary patterns may be valuable in addition to analysing relationships with single nutrients and food groups.

In summary, as discussed above, further investigation in larger cohorts, with well-defined BMI and body fat groups, using appropriate and valid taste perception measurements and dietary tools are needed to address this important knowledge gap in understanding the link between sweet and fat taste perception, dietary intake and metabolic health.

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## Chapter 3

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### ***Is Sweet Taste Perception Associated with Sweet Food Liking and Intake?***

Data published in: Jayasinghe, S., Kruger, R., Walsh, D., Cao, G., Rivers, S., Richter, M., & Breier, B. (2017). Is sweet taste perception associated with sweet food liking and intake? *Nutrients*, 9(750), 1-19. <https://doi.org/10.3390/nu9070750>

#### **3.1 Abstract**

A range of psychophysical taste measurements are used to characterise an individual's sweet taste perception and to assess links between taste perception and dietary intake. The aims of this study were to investigate the relationship between four different psychophysical measurements of sweet taste perception, and to explore which measures of sweet taste perception relate to sweet food intake. Forty-four women aged 20–40 years were recruited for the study. Four measures of sweet taste perception (detection and recognition thresholds, and sweet taste intensity and hedonic liking of suprathreshold concentrations) were assessed using glucose as the tastant. Dietary measurements included a four-day weighed food record, a sweet food-frequency questionnaire and a sweet beverage liking questionnaire. Glucose detection and recognition thresholds showed no correlation with suprathreshold taste measurements or any dietary intake measurement. Importantly, sweet taste intensity correlated negatively with total energy and carbohydrate (starch, total sugar, fructose, glucose) intakes, frequency of sweet food intake and sweet beverage liking. Furthermore, sweet hedonic liking correlated positively with total energy and carbohydrate (total sugar, fructose, glucose) intakes. The present study shows a clear link between sweet taste intensity and hedonic liking with sweet food liking, and total energy, carbohydrate and sugar intake.

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## 3.2 Introduction

Taste or gustation is the sensation experienced when a substance in the mouth is recognised by taste receptors of taste buds on the tongue papillae. There are five established basic tastes; sweet, salty, sour, bitter and umami (savoury) (Chandrashekar *et al.*, 2006). These taste sensations influence the consumption of food (sweet, salty, umami) and may trigger the rejection of toxins (bitter, sour). Thus, taste perception influences food selection and dietary intake. Furthermore, over the past few decades there has been an increasing interest in understanding the role taste perception plays in satiety, energy balance and long-term health (Donaldson *et al.*, 2009; Drewnowski, 1995). Emerging data suggests that the gustatory system may also be involved in many other important metabolic processes such as energy homeostasis and appetite regulation, thereby influencing body weight and health (Hevezi *et al.*, 2009; Martin *et al.*, 2009). Given that sweet taste has a powerful hedonic appeal, preferences for sweet foods and beverages are important contributors of body weight and obesity development (Malik *et al.*, 2006; Te Morenga *et al.*, 2013). Obesity is a global health issue of epidemic proportion (World Health Organisation, 2000), and interventions to halt the epidemic have been unsuccessful (Ng *et al.*, 2014; New Zealand Medical Association, 2014). Although the causes of obesity are complex, key drivers include over-consumption of inexpensive, highly palatable, energy-dense and nutrient-poor foods and beverages high in added sugar (Swinburn *et al.*, 2011). Global sugar intake (e.g., sucrose and high-fructose corn syrup), largely in the form of sweetened foods and beverages, has increased over the last few decades and has paralleled the increase in obesity rates (Baker and Friel, 2014; Popkin and Nielsen, 2003).

A range of different psychophysical measurements of sweet taste is used to characterise distinct aspects of sweet taste perception (Webb *et al.*, 2015; Drewnowski *et al.*, 1999). A widely used method involves threshold testing, which determines the responsiveness of a person to sweet taste stimuli. Sweet taste thresholds can assess either the detection or the recognition threshold, measuring the lowest concentration of a sweet tastant that can be detected or recognised as sweet respectively (Lawless and Heymann, 1999). Another commonly used psychophysical measurement of sweet taste perception is sweet taste intensity (Holt *et al.*, 2000). Using concentrations above the taste recognition threshold, referred to as suprathreshold concentrations, sweet taste intensity measures the sensation of intensity that is produced by the tastant at a given concentration (Lawless and Heymann, 1999). In addition to sweet taste intensity, a hedonic measure of liking or preference is used to characterise the hedonic value of the sweet taste perception (Drewnowski *et al.*, 1999).

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It is well known that taste has an important influence on food choices (Kourouniotis *et al.*, 2016; Honkanen and Frewer, 2009). Furthermore, there are large variations in sweet taste perception between individuals (Reed and McDaniel, 2006), which in turn could influence their specific dietary intake. For example, individuals who have a high sweet detection or recognition threshold, and/or a lower level of perceived sweet taste intensity, may require higher quantities of a sweet tastant to be satisfied with the perception of sweet taste they are experiencing. In addition, an increased hedonic liking of sweetness at high concentrations may result in increased consumption of sweet food (Duffy *et al.*, 2009).

A number of previous studies that have investigated the relationship between sweet sensitivity (assessed using sweet taste thresholds or sweet taste intensity measurements), or hedonic liking, and food intake have reported contradicting results. While most studies reported no relationship between sweet taste sensitivity (i.e., sweet taste thresholds or sweet taste intensity measurements) and the type or the amount of sweet food consumed (Holt *et al.*, 2000; Mahar and Duizer, 2007; Cicerale *et al.*, 2012; Martinez-Cordero *et al.*, 2015; Low *et al.*, 2016), one study showed a negative correlation between sweet (aspartame) recognition thresholds and energy intake (Martinez-Cordero *et al.*, 2015). Furthermore, a positive correlation was observed between sweet taste intensity of high intensity sweeteners stevia and sucralose, and mean total energy intake (Low *et al.*, 2016). Other studies have shown that a high hedonic liking or a strong preference for sweet taste was associated with a higher habitual intake of sweet food (Holt *et al.*, 2000), an increased consumption of sweet beverages (Mahar and Duizer, 2007), and the sugar content of preferred sugar-rich cereals (Mennella *et al.*, 2011). It is likely that discrepancies between studies due to differences in the study participants (gender, ethnicity, age), assessment methods of sweet taste perception (psychophysical measurement, type of sweet stimuli) or dietary intake assessment methods (food record, FFQ), generate inconsistencies about the potential biological or functional relationships. The increasing interest in the role of sweet taste perception and its influence on sweet food intake has led to a proliferation of studies using a range of sweet taste measurements including taste thresholds, intensity and hedonic liking (Holt *et al.*, 2000; Low *et al.*, 2016; Mattes and Mela, 1986). Many of these measurements, however, have not been rigorously assessed and may limit the value of the data obtained. Furthermore, there is a dearth of data that systematically compare the outcomes of different sweet taste perception measurements to each other and how they might relate to dietary intake and eating behaviour (Webb *et al.*, 2015; Low *et al.*, 2016; Mattes, 1985).

The present study had the overall goal to increase our understanding of the biological and functional links between sweet taste perception and sweet food intake. The specific aims were;

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firstly, to evaluate and compare four widely used psychophysical measurements that characterise sweet taste perception; and secondly, to explore whether any of these assessments of sweet taste perception may relate to sweet food intake. A better understanding of the different assessment methods of sweet taste perception, and a thorough evaluation of their relationships to each other and with sweet food intake will provide new insights into the role that sweet taste perception plays in habitual sweet food liking and intake, and how it may influence long-term health.

### **3.3 Methods**

#### **3.3.1 Study participants**

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was reviewed and approved by the Massey University Human Ethics Committee. All participants provided written informed consent prior to participating in the study. Forty-four healthy NZE women, aged 20–40 years, were recruited from the wider Auckland area. Participants were excluded if pregnant or breastfeeding, currently on any diet that excludes food groups (e.g., vegan), smoking or in the process of quitting, diagnosed with any metabolic illness, have conditions that could alter gustatory functions (e.g., chemotherapy), have any form of oral or nasal disease, currently taking medication that may influence taste perception or saliva production, or have taken antibiotics in the past three months (Steinbach *et al.*, 2009).

#### **3.3.2 Study procedure**

Participants visited the Human Nutrition Research Unit and Sensory Research Facilities at Massey University in Auckland on four separate sessions, at least a day apart and within a month (mean  $\pm$  SD: 21 $\pm$ 11 days). Four psychophysical measurements of sweet taste were determined at each session using glucose as the sweet tastant. These included; detection threshold, recognition threshold, sweet taste intensity and hedonic liking. Glucose was used as the sweet tastant as it is a simple sugar and has clearly defined metabolic links with glucose metabolism (Aronoff *et al.*, 2004). Furthermore, there is new evidence that show glucose sensors and ATP-gated K<sup>+</sup> channels within the taste cells involved in type 1 taste receptor-independent detection of sweet taste (Yee *et al.*, 2011). In addition, participants completed a four-day weighed food record, sweet food-food frequency questionnaire (SF-FFQ) and a sweet beverage liking questionnaire pertaining to food intake and sweet beverage liking. All four sessions were standardised by conducting the taste evaluation between 7–10 a.m. after an overnight fast (Nakamura *et al.*, 2008) to ensure sweet taste perception measurements were not influenced

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by hormonal fluctuations and different levels of hunger. Participants were asked to abstain from brushing their teeth one hour prior to testing, and were tested during any stage of their menstrual cycle (Nakamura *et al.*, 2008; Bryant *et al.*, 2006). At the first session participants completed a health and demographic questionnaire, and their height (stadiometer), weight and body fat percentage (Bioelectrical Impedance Assessment, InBody 230, Biospace, Cerritos, California, United States) were measured.

### **3.3.3 Sweet taste perception measurements**

At each of the four sessions, participants first completed the psychophysical measurements of glucose detection and recognition thresholds, followed by rating the sweet taste intensity and hedonic liking of four suprathreshold glucose concentrations. Sweet solutions were prepared on the day of testing by dissolving glucose (dextrose monohydrate, Qinquangdao Lihua Starch Co. Ltd., Qinquangdao, China) in distilled water. Sensory testing was conducted in individual sensory booths at room temperature (20° C). Participants tasted the samples using the whole mouth testing (sip and spit) method (Martinez-Cordero *et al.*, 2015), and did not wear nose clips during sensory testing.

#### **3.3.3.1 Glucose detection and recognition thresholds**

Eight glucose concentrations, ranging between 15–180 mM (15, 30, 45, 60, 90, 120, 150, 180 mM) were prepared to determine detection and recognition thresholds. This concentration range covered the published glucose detection and recognition thresholds (Nakamura *et al.*, 2008; Ileri-Gurel *et al.*, 2012), and allowed for inter-individual threshold differences. Participants received the samples at each concentration as a 3-alternative forced-choice ascending series (Chevrot *et al.*, 2014). Starting from the lowest glucose concentration, three samples (10 ml each), one containing the glucose sample and two background samples (distilled water) were presented at each concentration. Participants were asked to take the whole 10 ml of sample in their mouth, swirl the solution around for 3 seconds and expectorate. Using the forced-choice method, participants selected the sample with the sweet taste. Participants evaluated each glucose concentration three times at the same concentration level before moving on to the next higher concentration, completing 24 trials in total at each of the four sessions. All samples were given a three-digit number, and the position of the sweet sample was randomly allocated. Participants were asked to rinse their mouth with distilled water between each concentration step.

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### **3.3.3.2 Sweet taste intensity and hedonic liking of suprathreshold glucose concentrations**

Each participant rated the sweet taste intensity and hedonic liking of 125, 250, 500 and 1000 mM glucose samples (10 ml each) presented in a random order. Sweet taste intensity was rated on a 100 mm gLMS ranging from no sensation (0 mm) to strongest imaginable sensation (100 mm) with verbal descriptors assigned to different levels of intensities (Green *et al.*, 1996). Hedonic liking was rated on a hedonic gLMS ranging from dislike extremely (-100 mm) to like extremely (+100 mm) with neutral (0 mm) in the middle (Lim *et al.*, 2009). Participants were instructed and trained on how to use both gLMS scales according to the protocol outlined by Green *et al.* (1996) (Green *et al.*, 1996), and encouraged to mark anywhere on the scale in accordance with the level of taste perception they experienced. For example, if the sweet taste intensity of a sample was perceived between moderate and strong, they were asked to mark closer to the verbal descriptor that more closely represented the sweet sensation perceived.

### **3.3.4 Dietary measurements**

#### **3.3.4.1 Food intake**

Each participant completed a four-day weighed food record to assess their food intake (Gibson, 2005; Biro *et al.*, 2002). Participants were given verbal instructions and shown a food record video on how to maintain a weighed food record. They were instructed to record what was consumed, time of consumption, quantity consumed and how the food was prepared. All participants were given a food record booklet, electronic scales and a food portion booklet containing portion sizes (used when scales cannot be used e.g., dining out). Participants recorded their dietary intakes on four days consisting of one weekend day and a maximum of two consecutive days. On each of the testing sessions, the previous day's food record was checked for accuracy and completeness.

#### **3.3.4.2 Frequency of sweet food and beverage intakes**

The SF-FFQ was specifically developed to assess the frequency of sweet food and sweetened beverage consumption over the previous month (Appendix 3) (Low *et al.*, 2016). The main purpose of the SF-FFQ was to assess the habitual intake (in terms of frequency) of sweet tasting foods rather than quantifying their intake. The sweet foods included in the questionnaire were based on the 1997 and 2008/2009 New Zealand National Nutrition Surveys (Ministry of Health, 2011; Ministry of Health, 1999). Participants indicated their frequency of intakes of 69 sweet tasting foods and beverages (both natural sweet tasting foods and processed sweetened foods)

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using the following frequencies: never, less than once a month, 2–3 times per month, once per week, 2–4 times per week, 4–6 times per week, once a day, and twice or more a day (Daly *et al.*, 2011; Ireland *et al.*, 1994).

### **3.3.4.3 Sweet beverage liking**

A sweet beverage liking questionnaire was developed to measure hedonic preferences of 16 sweet tasting beverages (Duffy *et al.*, 2009). The sweet beverages included in the questionnaire were based on data from the 1997 and 2008/2009 national nutrition surveys, where beverages were listed as major sources of sugar intake of New Zealanders (Ministry of Health, 2011; Ministry of Health, 1999). Participants rated the liking of the sweet beverages on a 100 mm VAS anchored between strong dislike and strong like. Pictures and examples of the sweet beverages were available to assist participants in completing the task.

## **3.3.5 Data handling and statistical methods**

### **3.3.5.1 Sweet taste perception measurements**

Glucose detection and recognition thresholds were determined from the sweet taste threshold detection curves produced for each participant by graphing the probability of detection levels against the glucose concentrations. A best-fit curve for each participant was then fitted using a logistic regression model. This method was a modified graphical approach similar to that used by Lawless 2010 (Lawless, 2010). As the response variable (success or failure to choose the correct sample with the sweet taste) is binomial, a logistic regression model was used to predict the probability that each individual would successfully detect the sweet sample at each concentration. The model used a common intercept for all participants, but the slopes were estimated separately for each participant based on their results from the four sessions. The values of the fitted parameters (slopes and intercepts) estimated for each participant calculated for a range of concentrations was used to create sweet taste detection curves. Using the sweet taste detection curves, glucose concentrations (x-axis) corresponding to different probability of detection levels (y-axis) were interpolated to determine individual glucose thresholds. Interpolation at a probability of 0.8 was chosen as the detection threshold to represent threshold values obtained if a predetermined stopping rule of three consecutive correct identifications at one concentration was used (Kulkarni and Mattes, 2013). Interpolation at 0.99 probability of detection was chosen to represent recognition threshold.

Sweet taste intensity ratings were measured from 0 to 100 mm and hedonic liking ratings from –100 mm (dislike extremely) to +100 mm (like extremely). The average detection and

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recognition thresholds, sweet taste intensity and hedonic liking measurements over the four repeated sessions were used to explore relationships between sweet taste measurements, and between sweet taste and dietary measurements.

### **3.3.5.2 Dietary measurements**

All weighed food record data were entered into FoodWorks 7 (FoodWorks Professional 2013, Xyris Software), using the New Zealand Food Composite Database to determine total energy and macronutrient intakes. Carbohydrate intakes were differentiated into starch (polysaccharides) and total sugars (free and added mono- and di-saccharides). Participants whose daily energy intakes were outside 1000–5000 kcal (i.e., 4200–21,000 kJ) were excluded from the analysis (University of Otago and Ministry of Health, 2011; Willett, 2013). A total of 43 participants completed food records of which two participant's food records were excluded from the analysis due to under-reporting (<1000 kcal).

The SF-FFQ intakes of the 69 food items were converted to daily frequency equivalents (DFEs) calculated by allocating proportional values to the original frequency categories with reference to a base value of 1, equivalent to once a day (Daly *et al.*, 2011; Ireland *et al.*, 1994). The scores were calculated as follows: DFE score of 2—twice a day or more; 1—once per day; 0.71—4–6 times per week; 0.3—2–4 times per week; 0.14—once per week; 0.08—2–3 times per month; 0.03—less than once a month; 0—never. The 69 food items were categorised into eight main sweet food categories; fruit, vegetables, dairy, spreads/sweeteners, cereals, baking/sweets, desserts and beverages, and a median DFE score for each category was determined. Finally, a single DFE score for the frequency of total sweet food intake was also calculated.

The beverage liking questionnaire was assessed by measuring a liking score (in mm) from zero for each sweet beverage ranging from strong dislike (-50 mm) to strong like (+50 mm).

### **3.3.5.3 Statistical analysis**

All measurements related to sweet taste threshold detection curves were conducted using the R statistical program version 3.2.5. All other data analysis was conducted using SPSS software version 23 (IBM Corporation, New York, USA). All continuous variables were tested for normality using Kolmogorov-Smirnov test together with analysing histograms, normal Q-Q plots and boxplots. Non-normal data were log transformed and re-tested for normality. Parametric data are presented as mean  $\pm$  standard deviation (SD), and non-parametric data as median [25,75 percentiles]. Log transformed data are reported as mean [95% confidence intervals] (CI). Intraclass correlation coefficient (ICC) (two-way random effects model, absolute agreement,

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average measures) was used to assess the between-session correlation of thresholds, sweet taste intensity and hedonic liking over the four repeated sessions. An ICC value >0.7 was considered good correlation, while a ICC <0.7 was considered moderate to low correlation (Newman and Keast, 2013). Repeated measures design was used to test differences in sweet taste intensity and hedonic liking ratings between glucose concentrations. Relationships between two continuous variables were tested using the Pearson's correlation coefficient for parametric data and Spearman's correlation coefficient for non-parametric data. Correlation coefficients were used to determine the strength of the relationship by the criteria;  $\pm 0.1$  = weak,  $\pm 0.3$  = moderate and  $\pm 0.7$  = strong (Linneman, 2011). A  $p < 0.05$  was considered statistically significant. All statistical tests were 2-tailed.

## **3.4 Results**

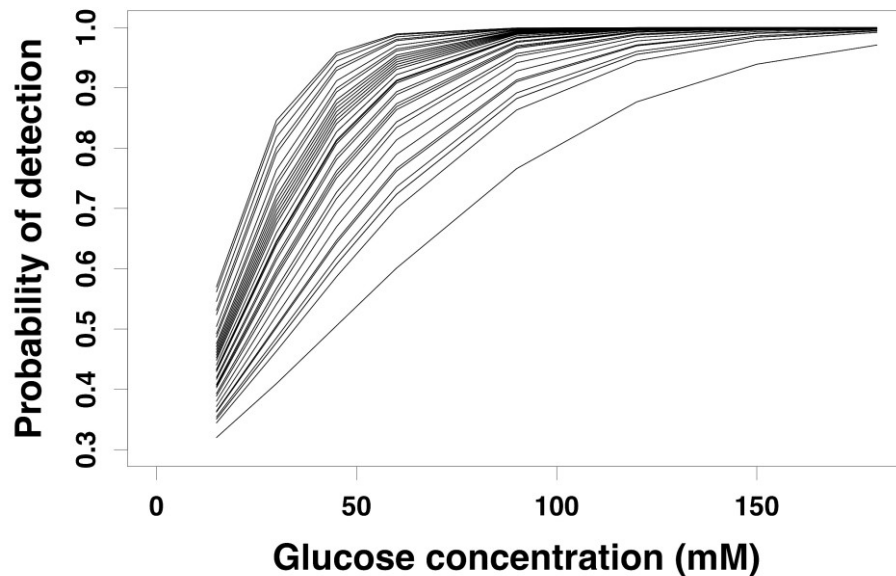
### **3.4.1 Participant characteristics**

Participants had a mean ( $\pm$  SD) age of  $28 \pm 6$  years and a mean [95% CI] body weight of 66.6[63.8, 69.5] kg. The mean [95% CI] BMI and body fat were 24.0[23.0, 25.1] kg/m<sup>2</sup> and 30.2[28.2, 32.2]% respectively.

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### 3.4.2 Sweet taste threshold detection curves

Figure 3.1 shows the sweet taste threshold detection curves of each participant describing the sweet taste detection at different glucose concentrations. The detection curves were produced by calculating the probability of sweet taste detection against the glucose concentrations using data from all four sessions. A binomial logistic regression model was used to determine the best-fit curve for each participant and interpolate glucose detection and recognition thresholds.



**Figure 3.1 Sweet taste threshold detection curves of all participants**

Each line of this figure represents the best-fit curve of each participant generated by a binomial regression model with a common intercept and separate slopes. The figure was generated from the average threshold data of the four repeated sessions.  $n=44$ .

As expected, all participants showed increased sweet taste detection with increasing glucose concentrations, which was observed across all four sessions. However, the rate of sweet detection, identified as the concentration at which each participant was able to consistently select the sweet sample, differed markedly between participants. The specific rate of detection for each participant was used as a marker of sweet sensitivity to interpolate glucose thresholds. Each participant's detection and recognition thresholds were determined by interpolating the glucose concentrations corresponding to different probability of detection levels using their own detection curve. Interpolation at a probability of detection of 0.8 was chosen as the detection threshold, and a probability of detection of 0.99 was chosen as recognition threshold.

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### 3.4.3 Glucose detection and recognition thresholds

The group median detection and recognition thresholds at each of the four sessions are reported in Table 3.1. The repeatability of glucose thresholds over the four sessions, tested using intraclass correlation coefficient, indicated strong between-session correlations (Table 3.1).

**Table 3.1 Glucose detection and recognition thresholds at the four different sessions**

	Detection threshold (mM)	Recognition threshold (mM)
Session 1	40.1[30.5, 62.3]	84.6[64.4, 131.4]
Session 2	40.6[37.7, 47.8]	90.2[83.8, 106.4]
Session 3	41.0[36.7, 49.3]	93.7[83.8, 112.7]
Session 4	40.8[36.9, 45.9]	90.1[81.5, 101.5]
Median of all sessions	41.3[38.7, 51.1]	91.0[85.5, 111.6]
ICC average measures <sup>a</sup>	0.64[0.43, 0.79]	0.67[0.47, 0.80]

ICC: intraclass correlation coefficient. All threshold data are interpolated from the sweet taste threshold detection curves and reported as median [25, 75 percentiles]. ICC values reported as mean [95% CI].

<sup>a</sup>  $p < 0.001$  for all between-session ICC measurements.  $n = 44$ .

### 3.4.4 Sweet taste intensity and hedonic liking at suprathreshold concentrations

The group mean sweet taste intensity and hedonic liking ratings of the four glucose concentrations at each of the four sessions are reported in Table 3.2. As expected, at each session, participants perceived the sweet taste as more intense with increasing glucose concentrations. Overall, across all sessions there was no significant difference in hedonic liking between 125 mM and 250 mM glucose, but a significant decrease in hedonic liking was observed at the higher glucose concentrations, 500 mM and 1000 mM glucose (Table 3.2). The between-session repeatability of sweet taste intensity and hedonic liking, assessed by intraclass correlation coefficient, increased as the glucose concentrations increased, with sweet taste intensity and hedonic liking of 1000 mM glucose showing the highest between-session ICC (Table 3.2).

**Table 3.2 Sweet taste intensity and hedonic liking ratings at the four different sessions**

<b>Sweet Taste Intensity (mm)</b>				
	125 mM	250 mM	500 mM	1000 mM
Session 1	10±9	23±15	48±19	67±17
Session 2	7±6	19±11	40±16	64±18
Session 3	8±6	21±15	47±19	66±16
Session 4	8±10	19±14	45±24	70±18
Average of all sessions	9±8	21±11	46±16	67±14
ICC average measures <sup>a</sup>	0.65[0.44, 0.80]	0.61[0.38, 0.78]	0.81[0.70, 0.90]	0.84[0.74, 0.91]

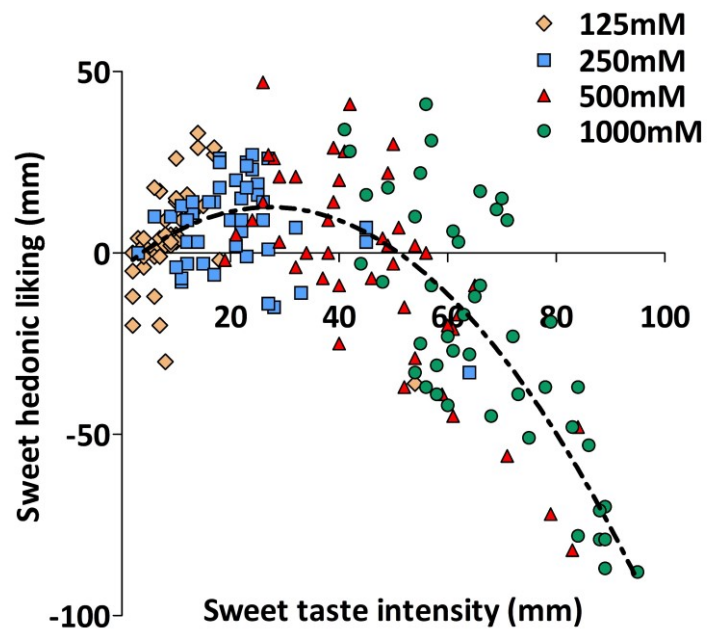
<b>Sweet Hedonic Liking (mm)</b>				
	125 mM	250 mM	500 mM	1000 mM
Session 1	4±19	7±19	-5±28	-20±34
Session 2	4±18	7±15	-1±30	-22±38
Session 3	4±21	9±20	-2±39	-17±45
Session 4	4±17	7±19	-7±35	-32±41
Average of all sessions	4±14	8±13	-4±28	-23±35
ICC average measures <sup>a</sup>	0.78[0.65, 0.87]	0.67[0.48, 0.81]	0.88[0.81, 0.93]	0.90[0.84, 0.94]

ICC: intraclass correlation coefficient. Sweet taste intensity and hedonic liking reported as mean ± SD. ICC values reported as mean [95% CI]. <sup>a</sup>  $p < 0.001$  for all between-session ICC measurements.  $n = 44$ .

The suprathreshold sweet taste intensity and hedonic liking ratings at any glucose concentration did not correlate with glucose detection or recognition thresholds. However, the relationship between sweet taste intensity and hedonic liking showed a concentration-dependent bi-phasic relationship.

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Figure 3.2 shows the scatterplot of the relationship between sweet taste intensity and hedonic liking across all four glucose concentrations generated using the average ratings across the four repeated sessions. Importantly, the relationship between sweet taste intensity and hedonic liking changed with increasing glucose concentrations. A positive relationship was present at the lowest glucose concentration 125 mM ( $r_s=0.52$ ,  $p<0.001$ ), followed by no relationship at 250 mM, and shifting to a clear negative relationship at the two highest concentrations, 500 mM ( $r=-0.75$ ,  $p<0.001$ ) and at 1000 mM ( $r=-0.76$ ,  $p<0.001$ ).



**Figure 3.2 Scatterplot of the relationship between sweet taste intensity and hedonic liking**  
The scatterplot was generated using the average sweet taste intensity and hedonic liking ratings across the four repeated sessions.  $n=44$ .

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### 3.4.5 Food intake

The study population's mean daily intakes of total energy, macronutrients and total sugars, obtained using four-day weighed food records are reported in Table 3.3. Intakes of macronutrients and sugars are expressed as absolute intakes in grams and as a percentage of total energy intake. The mean energy intake of the group (7698 kJ) was slightly lower than the estimated energy requirement (8000–8400 kJ) for women of similar age and weight (National Health and Medical Research Council, 2006). With reference to the AMDR for carbohydrate (45–65%), protein (15–25%) and fat (20–35%) (National Health and Medical Research Council, 2006), the average intakes of this group were slightly lower for carbohydrate intake (42%) and higher for fat intake (37%). Furthermore, sucrose was the predominant sugar subgroup contributing to 8% of total energy intake.

**Table 3.3 Daily intakes of total energy, macronutrients and sugars**

Energy/Nutrients	Intake
Total energy (kJ)	7698.0±1716.9
Protein (g)	78.9±18.4
Protein (%) <sup>a</sup>	17.8±4.0
Fat (g)	77.4±22.1
Fat (%) <sup>a</sup>	37.2±7.2
Carbohydrate (g)	189.6±62.3
Carbohydrate (%) <sup>a</sup>	41.7±8.6
Starch (g)	100.7±32.5
Starch (%) <sup>a</sup>	22.2±5.3
Total Sugar (g) <sup>b</sup>	88.9±38.0
Total sugar (%) <sup>a</sup>	19.4±6.1
Sucrose (g)	38.8±21.7
Sucrose (%) <sup>a</sup>	8.3±3.5
Fructose (g)	18.9±9.2
Fructose (%) <sup>a</sup>	3.9±1.7
Glucose (g)	17.6±8.6
Glucose (%) <sup>a</sup>	4.2±2.2
Lactose (g)	11.3±7.1
Lactose (%) <sup>a</sup>	2.5±1.5
Maltose (g)	2.8±1.6
Maltose (%) <sup>a</sup>	0.6±0.3

All data were obtained from the four-day weighed food records and reported as mean ± SD. <sup>a</sup> Calculated as a % of total energy intake. <sup>b</sup> Total sugars include all mono- and di-saccharides. *n*=41.

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### 3.4.6 Frequency of sweet food intake

Table 3.4 shows the frequency of daily intakes of the eight sweet food categories assessed using the SF-FFQ. On average, each participant consumed a combination of naturally sweet foods and foods with added sugars or sweeteners seven times a day. The three most frequently consumed food categories were fruit, baking/sweets and sweet beverages, and the least consumed sweet categories were sweet vegetables and desserts.

**Table 3.4 Frequency of intake of sweet food categories**

Food Category	DFE <sup>a</sup>
Fruit (e.g., bananas, apples, dried fruit)	1.8[1.1, 3.4]
Baking/sweets (e.g., chocolates, biscuits, cakes)	1.2[0.6, 1.7]
Beverages (e.g., fruit juice, soft drinks, fruit smoothies)	0.8[0.3, 1.1]
Dairy (e.g., yoghurt, flavored milk, yoghurt drinks)	0.6[0.2, 1.1]
Cereals (e.g., muesli, liquid breakfasts, cereals)	0.4[0.1, 0.9]
Spreads/sweeteners (e.g., sugar, jam, honey/golden syrup)	0.3[0.1, 0.9]
Vegetables (e.g., kumara, beetroot, pumpkin)	0.2[0.2, 0.6]
Desserts (e.g., ice cream, custard, jelly)	0.2[0.1, 0.2]
Total sweet food	7.1±3.0

DFE: daily frequency equivalent. Total sweet food reported as mean ± SD. All other data reported as median [25, 75 percentiles]. <sup>a</sup> DFE score of 2—twice a day or more; 1—once per day; 0.71—4–6 times per week; 0.3—2–4 times per week; 0.14—once per week; 0.08—2–3 times per month; 0.03—less than once a month; 0—never. *n*=44.

### 3.4.7 Sweet beverage liking

The mean liking scores for all 16 sweet beverages are reported in Table 3.5. The three most liked sweet beverages were fruit smoothies, cocktails and dessert wine/ciders. The least liked drinks were fruit drinks, cordials and energy drinks.

**Table 3.5 Liking scores of sweet beverages**

Sweet Beverage	Liking Score (mm)
Fruit Smoothie	24.2±19.2
Cocktail	13.8±27.1
Dessert wine/Cider	10.9±29.3
Milk mixer	10.3±22.4
Fruit Juice	8.8±23.4
Iced coffee	1.8±37.0
Flavored milk/Milkshakes	1.3±30.1
Iced tea	-2.2±29.9
Soft drink (regular)	-3.0±28.3
Flavored water	-4.8±24.5
Spirits	-6.0±28.4
Soft drink (sugar free)	-6.6±28.4
Yoghurt drink	-7.1±28.5
Fruit drink	-8.2±25.2
Cordial	-18.2±22.1
Energy drinks	-23.2±26.4

All data generated from the sweet beverage liking questionnaire and reported as mean ± SD. *n*=44.

### 3.4.8 Relationship between sweet taste perception and dietary measurements

#### 3.4.8.1 Sweet taste perception and food intake

There was no correlation between sweet taste detection and recognition thresholds, and total energy intake and intakes of macronutrients and sugars in grams or expressed as a percentage of total energy ( $p>0.05$ ). The correlation between sweet taste intensity and energy, macronutrients and sugar intakes are reported in Table 3.6. There was a clear dose-dependent negative correlation between sweet intensity perceived at 250 mM, 500 mM and 1000 mM glucose, and total energy, and carbohydrate, starch, total sugar and glucose intakes in grams. However, sweet taste intensity at any glucose concentration was not correlated with macronutrients and sugars intakes when expressed as a percentage of total energy intake ( $p>0.05$ ).

**Table 3.6 Correlation coefficients of the relationship between sweet taste intensity and food intake**

Energy/Nutrients	Sweet Taste Intensity			
	125 mM	250 mM	500 mM	1000 mM
Total energy (kJ)	-0.19	<b>-0.38*</b>	<b>-0.36*</b>	<b>-0.40**</b>
Protein (g)	-0.24	-0.10	-0.21	-0.20
Fat (g)	-0.01	-0.18	-0.07	-0.19
Carbohydrate (g)	-0.24	<b>-0.42**</b>	<b>-0.43***</b>	<b>-0.45***</b>
Starch (g)	-0.28	<b>-0.42**</b>	<b>-0.40**</b>	<b>-0.41**</b>
Total Sugars (g) <sup>a</sup>	-0.22	<b>-0.35*</b>	<b>-0.36*</b>	<b>-0.38**</b>
Sucrose (g)	-0.15	-0.27	-0.26	-0.29
Fructose (g)	-0.28	-0.28	<b>-0.39*</b>	<b>-0.37*</b>
Glucose (g)	-0.3	-0.34*	<b>-0.41**</b>	<b>-0.41**</b>
Lactose (g)	-0.20	-0.30	-0.19	-0.18
Maltose (g)	0.04	-0.05	-0.22	<b>-0.32*</b>

Correlation coefficients determined by Pearson's correlation coefficient for parametric data and Spearman's correlation coefficient for non-parametric data. <sup>a</sup> Total sugars include all mono- and disaccharides. \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$ .  $n=41$ .

The correlations between sweet hedonic liking and intake of energy, macronutrients and sugars are reported in Table 3.7. A positive correlation was observed between hedonic liking of 500 mM and 1000 mM glucose, and total energy, carbohydrate, total sugar, fructose, glucose and maltose intakes in grams. However, when expressed as a percentage of total energy, only with intakes of total sugar and maltose were positively correlated with sweet taste intensity of 1000 mM.

**Table 3.7 Correlation coefficients of the relationship between sweet hedonic liking and food intake**

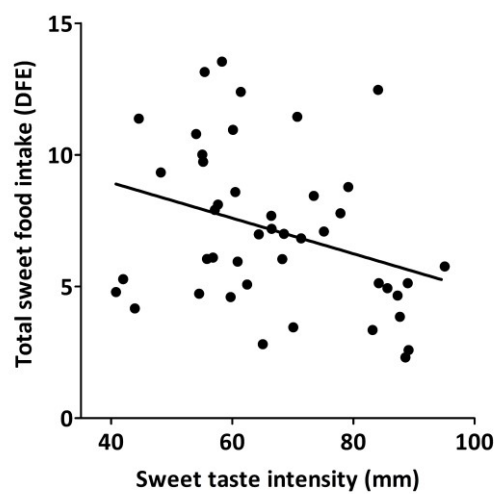
Energy/Nutrients	Sweet Hedonic Liking			
	125 mM	250 mM	500 mM	1000 mM
Total energy (kJ)	0.04	0.18	<b>0.31*</b>	<b>0.32*</b>
Protein (g)	-0.05	0.01	0.19	0.13
Fat (g)	-0.02	0.08	0.1	0.19
Carbohydrate (g)	0.003	0.13	<b>0.34*</b>	<b>0.36*</b>
Starch (g)	0.01	0.12	0.22	0.21
Total Sugars (g) <sup>a</sup>	-0.01	0.11	<b>0.37*</b>	<b>0.41**</b>
Sucrose (g)	-0.05	0.08	0.23	0.29
Fructose (g)	-0.01	0.06	<b>0.33*</b>	<b>0.35*</b>
Glucose (g)	-0.06	0.02	<b>0.39*</b>	<b>0.42**</b>
Lactose (g)	0.01	0.12	<b>0.31*</b>	0.27
Maltose (g)	0.08	0.14	<b>0.40**</b>	<b>0.46**</b>

Correlation coefficients determined by Pearson's correlation coefficient for parametric data and Spearman's correlation coefficient for non-parametric data. <sup>a</sup> Total sugars include all mono- and di-saccharides. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .  $n = 41$ .

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### 3.4.8.2 Sweet taste perception and frequency of sweet food intake

No significant correlations were found between detection threshold, recognition threshold or hedonic liking, and the frequency of intake of any sweet food category. However, there was a negative correlation between the frequency of baking/sweets intake and sweet taste intensity perceived at 125 mM ( $r_s=-0.44$ ,  $p=0.003$ ), 250 mM ( $r_s=-0.43$ ,  $p=0.003$ ), 500 mM ( $r_s=-0.37$ ,  $p=0.01$ ) and 1000 mM ( $r_s=-0.31$ ,  $p=0.04$ ) glucose. Furthermore, perceived sweet taste intensity of 500 mM and 1000 mM glucose was negatively associated with the frequency of total sweet food intake ( $r=-0.33$ ,  $p=0.03$ , Figure 3.3).

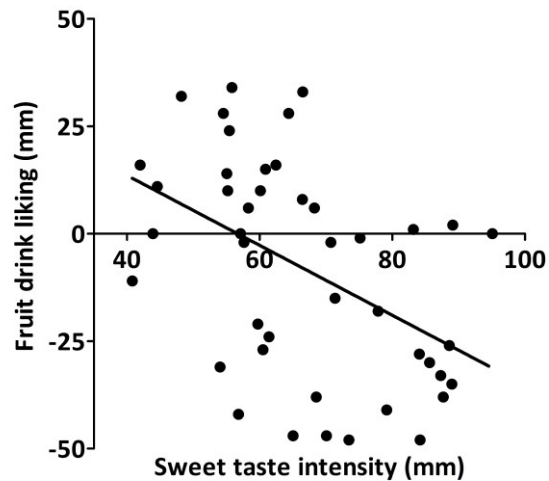


**Figure 3.3 Relationship between sweet taste intensity and sweet food intake**

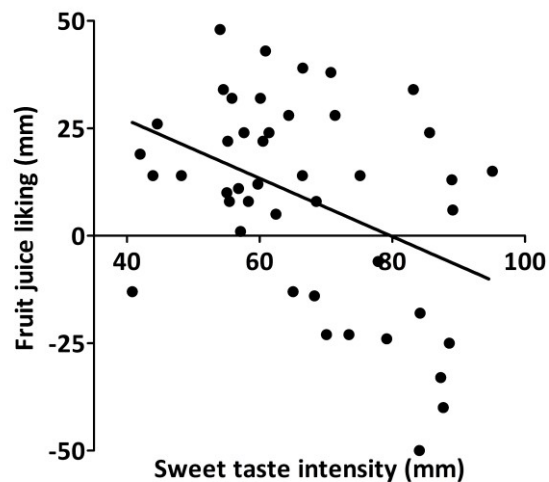
The above graph shows the relationship between sweet taste intensity of 1000 mM glucose and frequency of total sweet food intake ( $r=-0.33$ ,  $p=0.03$ ). DFE: daily frequency equivalent.  $n=44$ .

### 3.4.8.3 Sweet taste perception and sweet beverage liking

Correlation analysis showed that liking of fruit drink was negatively correlated with perceived sweet taste intensity of 500 mM ( $r=-0.32, p=0.03$ ) and 1000 mM ( $r=-0.42, p=0.005$ , Figure 3.4a), and positively correlated with sweet hedonic liking of 500 mM ( $r=0.35, p=0.02$ ), and 1000 mM glucose ( $r=0.34, p=0.02$ ). Furthermore, liking of fruit juice was negatively correlated with the perceived intensity of 500 mM and 1000 mM glucose ( $r=-0.47, p=0.001$ , Figure 3.4b).



a.



b.

**Figure 3.4 Relationship between sweet taste intensity and sweet beverage liking**

The above graph shows the relationship between sweet taste intensity of 1000 mM glucose and fruit drink liking (a) ( $r=-0.42, p=0.005$ ) and fruit juice liking (b) ( $r=-0.47, p=0.001$ ).  $n=44$ .

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## 3.5 Discussion

The present study evaluated the relationship between four widely used assessment methods of sweet taste perception and investigated the link between these sweet taste measurements and sweet food liking and intake. This paper had four main findings. Firstly, detection and recognition thresholds showed no correlations with perceived sweet taste intensity or hedonic liking. Secondly, a dose-dependent change in the relationship between sweet taste intensity and hedonic liking of suprathreshold concentrations indicated that a sweet tastant is liked at a lower concentration and disliked at higher concentrations in a dose-dependent manner. Thirdly, although individual participants showed distinct patterns of sweet detection, glucose detection and recognition thresholds were not correlated with intakes of energy, macronutrients and sugars, frequency of sweet food intake or sweet beverage liking. Lastly, total energy intake, and absolute intake of carbohydrate (i.e., starch, total sugar, fructose and glucose) correlated negatively with sweet taste intensity and positively with hedonic liking of suprathreshold glucose concentrations in a dose-dependent manner. To the best of our knowledge this is the first study to report robust relationships between sweet taste intensity and hedonic liking of suprathreshold glucose concentrations, and food intake and sweet beverage liking. These findings have implications for eating behaviour and long-term health outcomes, as sensory properties of foods and beverages clearly influence preferences, and the type and amount of food consumed.

### 3.5.1 Inter-individual variations in sweet taste perception measurements

In the present study, detection and recognition thresholds were interpolated from sweet taste threshold detection curves produced for each participant. This is the first study that determines glucose thresholds using the sweet taste threshold detection curve method. Although the detection of sweet taste of all participants increased with increasing glucose concentration, each participant had a distinct sweet detection curve, with a distinct rate of sweet taste detection. This indicates clear inter-individual variations in sweet sensitivity. The median glucose detection (41.3 mM) and recognition (91.0 mM) thresholds in the present study were similar to previously published mean detection and recognition thresholds of 54 mM (Ileri-Gurel *et al.*, 2012) and 95.3 mM (Nakamura *et al.*, 2008) respectively, but they were higher compared to another study with the reported mean glucose detection and recognition thresholds of 17.2 mM and 35.2 mM respectively (Low *et al.*, 2016).

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It is well known that methodological details associated with threshold testing contribute to the inherent variability of the data between different laboratories making it difficult to compare threshold values between studies. The stopping rule, which determines the point of termination of the taste testing, also influences the allocation of threshold values (Peng *et al.*, 2012). One of the strengths of the present study is that glucose thresholds obtained from the sweet taste threshold detection curves used no predetermined stopping rule and therefore did not rely on a stopping rule purely determined by chance (Lawless, 2010). As seen from the data of the present study, the detection of sweet taste does not change from no-detection (probability zero) to detection (probability one), but rather describes a gradual increase in detection with increasing concentrations. Therefore, by using each participant's dose-response curve, more accurate sweet taste thresholds can be determined. Furthermore, the intraclass correlation coefficient showed good between-session repeatability for detection (ICC=0.64) and recognition (ICC=0.67) thresholds, similar to the sucrose detection thresholds (ICC=0.66) reported in a previous study (Newman and Keast, 2013). This shows that glucose thresholds were consistent and reproducible over repeated sessions in this study.

In the present study, perceived sweet taste intensity increased with increasing glucose concentrations, suggesting that the glucose concentrations were able to clearly evoke different levels of sweet taste intensities. Furthermore, the hedonic liking ratings indicated that participant's liking of sweet taste was concentration-dependent. The current study found that the between-session correlations for both sweet taste intensity and hedonic liking increased with increasing glucose concentrations, similar to a previous study where higher repeatability of perceived pleasantness ratings were observed for solutions with higher sugar levels (Coulon *et al.*, 2012). This suggests that an individual's ability to consistently perceive the intensity or hedonic liking of a sweet solution is greater when the strength of the sweet signal is stronger (i.e., higher concentrations), possibly due to the increased saturation of the receptor-ligand interactions of sweet taste receptors (Nie *et al.*, 2005).

The inter-individual phenotypic variations in sweet taste thresholds, sweet intensity and hedonic liking observed in the present study could possibly be explained by the genetic variation of the sweet taste receptor T1R2 and T1R3 subunits of the G-protein coupled receptor responsible for sweet taste (Chamoun *et al.*, 2016). In particular, single nucleotide polymorphisms of the T1R2 and T1R3 receptors have been associated with variations in sweet taste perception and consumption of sweet foods (Eny *et al.*, 2010; Fushan *et al.*, 2009). Although genetic variation was not investigated in the present study, this emerging field of research could provide further understanding of the inter-individual differences in sweet taste perception.

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### 3.5.2 Relationship between sweet taste perception measurements

The present study found no correlation between the glucose thresholds, and sweet taste intensity or hedonic liking of any suprathreshold glucose concentration similar to previous studies using sweet, sour and salty, and caffeine tastants (Webb *et al.*, 2015; Mattes, 1985; Bartoshuk, 1978). These results suggest that a person with a low detection or recognition threshold for a sweet tastant may not necessarily experience a greater sweet sensation or like sweet taste at low concentrations. The lack of relationship between thresholds and suprathreshold measurements suggest that each psychophysical measurement characterises a specific feature of sweet taste. As already discussed by Webb *et al.* (2015), one sweet taste measure alone is not a convincing marker of overall sweet taste perception. Therefore, a combination of sweet taste measurements may provide a better understanding of the sense of taste, and enhance the inquiry about relationships between taste perception and food intake (Webb *et al.*, 2015).

One of the important findings of the present study describes the change in the relationship between sweet taste intensity and hedonic liking with increasing concentrations of sweet tastant, starting with a positive relationship at the lowest glucose concentration, and moving to a negative relationship at the two highest glucose concentrations. This relationship between sweet taste intensity and liking at the lowest (125 mM) glucose concentration shows that participants who perceived this concentration as sweet, liked the level of sweetness more than those who experienced the same concentration as less sweet. Importantly, participants who perceived the two highest glucose concentrations as more sweet disliked the sweetness more than participants who perceived the solutions as less sweet. This change in the relationship between sweet taste intensity and liking suggests that the intensity measurements at suprathreshold concentrations relate more strongly to the hedonic experience. Furthermore, the finding that participants generally disliked the two highest concentrations has implications for our food environment, because these levels of sugars or other sweeteners are commonly found in sweet beverages. Our study suggests that there is ample scope to reduce the sugar content in sugar-sweetened beverages but still maintain hedonic liking.

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### 3.5.3 Characterising food intakes of the study participants

The four-day weighed food record data indicated that the study participants on average consumed relatively low levels of carbohydrate, but high levels of fat, and moderate levels of protein. The mean energy intake of the group (7698 kJ) was slightly lower than the estimated energy requirement (8000–8400 kJ) for women of similar age and weight (National Health and Medical Research Council, 2006). Furthermore, total sugar (88.9 g) and sucrose (38.8 g) intakes were generally lower than the mean total sugar (97–121 g) and sucrose (43.6–61.6 g) intakes of a similar population in New Zealand (Ministry of Health, 2011). According to the SF-FFQ data from the present study, the most frequently consumed sweet food categories were fruit, baking/sweets and sweet beverages, and as indicated by the sweet beverage liking questionnaire, fruit smoothie was the most liked sweet beverage. Together these data indicate that the participants of the present study were consuming a relatively healthy diet with moderate intakes of fructose and glucose and relatively low intake of sucrose.

### 3.5.4 Suprathreshold taste measurements and food intake

To the best of our knowledge this is the first study to report robust significant relationships between sweet taste intensity and hedonic liking at suprathreshold concentrations of a sweet tastant, and food intake. Previous studies have failed to find associations between sweet taste and diet parameters (Drewnowski *et al.*, 1999; Martinez-Cordero *et al.*, 2015; Low *et al.*, 2016; Mattes, 1985). One of the strengths of the food intake data from the present study is that it was derived from in-depth weighed food records, which is considered the ‘gold standard’ method for quantifying nutrient intake (Biro *et al.*, 2002). The dose-dependent negative correlation between sweet taste intensity, and total energy intake and absolute intakes of carbohydrate (as well as starch, total sugar, fructose and glucose) in the present study suggests that participants who perceived glucose solutions as more sweet have lower energy, carbohydrate and sugar intakes in comparison with participants who perceived the glucose solutions as less sweet. This is supported by findings of a recent study that showed that reduced perceived intensity correlated with increased desire for higher calorie taste stimuli (Noel *et al.*, 2017).

The significant positive correlation between hedonic liking of 500 mM and 1000 mM glucose, and total energy and absolute intakes of carbohydrate (as well as total sugar, fructose, glucose) suggests that participants who have a higher hedonic liking for sweet taste consumed more energy, carbohydrates and especially more sugars. This positive association supports previous research where a positive relationship was found between hedonic liking of sweet taste, and

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liking ratings of sweet desserts and sugar in tea (Drewnowski *et al.*, 1999), frequency of sweet food consumption, intake of refined and total sugars (Holt *et al.*, 2000), and sugar content of favourite cereals (Mennella *et al.*, 2011).

Interestingly, this study found no relationship between sweet taste intensity and hedonic liking, and fat or protein intake, illustrating the clear link between sweet taste and intake of sweet tasting food. Furthermore, the lack of correlation between suprathreshold sweet measurements, and sucrose intake could be attributed to the generally lower intake of sucrose in this group of women. We also observed no correlations between sweet taste intensity and hedonic liking, and macronutrient intakes expressed as a percentage of total energy. Due to the significant relationship with total energy, it is not surprising that the relationship between sweet taste intensity and hedonic liking, and macronutrient intake were not significant when adjusted for total energy intake. A similar finding was reported by Stewart *et al.* (2011), where a significant difference in absolute intakes of fat and saturated fat between oleic acid hyper- and hypo-sensitive groups resulted in a loss of significant differences after adjusting fat intakes for total energy (Stewart *et al.*, 2011a). The same study suggested that because fatty acids interact directly with the taste receptors, the absolute intake of fat was more biologically relevant than the energy adjusted macronutrient intakes when assessing relationships between taste and food intake (Stewart *et al.*, 2011a). Nevertheless, the findings of our study illustrate an important biological relationship between how sweet taste is perceived and liked, and the intake of energy and macronutrients even within this group of young women with relatively healthy diets.

### **3.5.5 Suprathreshold taste measurements, frequency of sweet food intake and sweet beverage liking**

The data from the SF-FFQ and sweet beverage liking questionnaires showed a clear negative correlation between sweet taste intensity of 500 mM and 1000 mM glucose, and frequency of total sweet food intake (as well as the frequency of baking/sweets intake) and liking of fruit juice and fruit drink. This suggest that participants who perceived the highest glucose concentrations as more sweet are more sensitive to sweet taste and therefore had a lower frequency of sweet food intake and lower preferences for sweetened beverages compared to those who perceived the solution as less sweet.

The sweet beverage-liking questionnaire also showed that fruit drink liking was correlated positively with the hedonic liking of 500 mM and 1000 mM glucose, suggesting that participants who have a higher hedonic liking for sweet taste have increased liking for this sweet beverage. It is not surprising to find no other significant relationships between the hedonic liking of the

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glucose solutions and other sweet beverages, as the sensory experience of taste testing in a laboratory setting is different, and does not perfectly emulate sensory experiences of real-world beverages. For example, the sweet tastants in our study were present at room temperature while sweet beverages in the real-world are usually served cold to increase its palatability and sensory properties (Delwiche, 2004). We believe this contributed to the lack of relationship between hedonic liking of glucose solutions and liking of sweetened beverages.

### **3.5.6 Glucose thresholds, food intake and sweet beverage liking**

Although sweet taste threshold detection curves showed clear inter-individual variations in sweet taste detection, no correlations were found between glucose detection and recognition thresholds, and any dietary measurement. This finding is consistent with previous studies where no relationships were found between sweet taste thresholds and food intake (Martinez-Cordero *et al.*, 2015; Low *et al.*, 2016; Mattes, 1985). It has previously been discussed that taste thresholds are poor predictors of taste experienced with real world foods, because taste thresholds measure the lowest concentration of a tastant detected or recognised (Bartoshuk, 1978; Bartoshuk *et al.*, 2006). This phenomenon may also explain the lack of an association between sweet taste thresholds and dietary measurements observed in the present study.

### **3.5.7 Summary**

The present study shows significant relationships between dietary measurements and both sweet intensity and hedonic liking of suprathreshold concentrations, as previous studies have only found links between dietary measurements and sweet intensity (Low *et al.*, 2016) or between dietary measurements and hedonic liking (Holt *et al.*, 2000; Mahar and Duizer, 2007; Mennella *et al.*, 2011). The correlations found in this study can only establish a link between sweet taste intensity with increased sweet food intake, and cannot establish the direction of this relationship. It is possible that either a habitually high sweet food intake contributes to lower perception of sweet taste intensity, or, a lower sweet intensity perception may lead to increased habitual sweet food intake. However, this significant relationship is consistent with a recent study reporting sweet intensity to be the most appropriate measure to assess links between sweet taste and food intake (Low *et al.*, 2016). Furthermore, a recent dietary intervention study showed that participants rated pudding samples as sweeter following a three-month diet of reduced sugar intake, indicating sweet taste intensity to be an important factor associated with habitual food intake (Wise *et al.*, 2015). It is tempting to speculate that this relationship may be modifiable by dietary changes.

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The current food environment exposes individuals to strong cues that favour energy availability and a positive energy balance, which can lead to obesity and other metabolic disorders (e.g., type 2 diabetes) (Malik *et al.*, 2006; Te Morenga *et al.*, 2013). Commonly cited causes of obesity include major changes in our food environment which have led to over-consumption of highly palatable energy-dense, nutrient-poor and inexpensive foods with a noticeable increase in sugar intake over the past 30 years (Popkin and Nielsen, 2003; Duffey and Popkin, 2008). Our data suggest that sweet taste intensity and hedonic perceptions of suprathreshold concentrations may play a biological role in dietary intake (energy and carbohydrates), frequency of sweet food consumption and sweet beverage liking, thereby influencing body weight and long-term health. The nature (cause or effect) of this relationship requires further investigation.

The strengths of the present study include a thorough comparison of four commonly used psychophysical measurements of sweet taste perception, and an investigation of the relationship between all four sweet taste measurements with a range of parameters of sweet food intake. Furthermore, the range of dietary assessments investigated actual food intakes (food record), habitual intakes of sweet foods (SF-FFQ) and liking of sweet beverages, capturing different aspects of food intake and liking. The present study has several limitations that require further study. Firstly, participants of the study were a small sample of NZE women of similar age (young) and BMI (normal range). Therefore, the findings of this study cannot be generalised to other ethnicities, ages or BMI groups. Secondly, the study design was cross-sectional and the findings show only relationships and no causations can be ascertained. Thirdly, the participants of this study consumed a relatively healthy diet with generally low carbohydrate intakes and did not consume excessive amounts of sweet food. Therefore, the sample of dietary data in the present study may represent a relatively healthy spectrum of normal intakes. Lastly, the study used glucose as the sweet tastant and findings may differ if other sweet tastants are used.

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### 3.6 Conclusions

Our study has several important findings. The changing relationship between sweet taste intensity and liking with increasing glucose concentrations illustrates a clear relationship that is dependent on an individual's perception of sweet taste intensity. Furthermore, individuals who perceive glucose solutions as more sweet have lower intakes of energy and carbohydrate (starch, total sugars, fructose, glucose), as well as a lower frequency of sweet food intake and lower liking for sweet beverages compared to those who perceive the glucose solutions as less sweet. This notion is in agreement with a positive relationship between sweet hedonic liking and total energy and carbohydrate (total sugar, fructose, glucose) intakes, confirming that individuals who like the sweetness of the high glucose concentrations have higher habitual intakes of energy and sugars.

The present study shows a clear link between sweet taste intensity and hedonic liking, and dietary measurements in a group of young healthy women with normal BMI and relatively healthy food intakes. Stronger correlations between sweet taste and dietary measurements may exist in groups of women with higher variations of sweet food intake, especially if the sweet food intake contributes to an excess energy intake. Further research is needed to determine whether the relationships between sweet taste perception and food intake can be confirmed in other populations such as groups with different BMIs or people with healthy and unhealthy eating habits. Future studies should also employ objective measures of food intake in addition to the subjective measures used in our study. Furthermore, it is important to understand whether dietary interventions of reduced sweet food intake can change the perception of sweet taste intensity and liking. A better understanding of the relationship between sweet taste perception and dietary intake and eating behaviours will provide new insights into taste-related eating habits that may influence long-term health.

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## Chapter 4

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### ***Exploring the Link Between Sweet Taste and Fat (Creaminess) Perception, Body Composition and Metabolic Biomarkers***

#### **4.1 Abstract**

It is unclear whether a link between sweet and fat taste perception and obesity exists as published data are contradictory and inconclusive. The aims of our study were to investigate differences in sweet taste and fat (creaminess) perception between ethnic groups with known differences in metabolic disease and obesity risk profiles (NZE, Māori and Pacific) and across different body composition groups. Further, to assess the direct link between sweet taste and fat (creaminess) perception and metabolic and endocrine biomarkers of adiposity and appetite. A total of 408 NZE, Māori and Pacific women aged 16–45 years were recruited as part of the women’s EXPLORE study. Participants rated the sweet taste and fat (creaminess) intensity and hedonic liking of five sucrose and milk samples on a gLMS. Body composition groups were determined according to their BMI and body fat content. Metabolic and endocrine biomarkers associated with adiposity and appetite (e.g., glucose, insulin, leptin) were used to assess the relationship with taste perception. We observed no significant differences in sweet and fat (creaminess) perception (taste intensity or hedonic liking) between ethnic groups or body composition groups. Furthermore, apart from a few weak correlations, no robust direct relationships between sweet and fat perception and metabolic and endocrine biomarkers were found. Our data suggest that in this group of healthy pre-menopausal women, sweet taste and fat (creaminess) perception is not different between ethnic groups with known differences in metabolic disease and obesity risk profiles or across different BMI and body fat profiles. Larger trials within each ethnic group with a greater range of body fat profiles and in women or men with different metabolic health statuses (e.g., diabetes) should be conducted to support or challenge the current findings.

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## 4.2 Introduction

Significant changes in the global food system and physical activity patterns over the last three decades have been linked with increased rates of obesity and associated co-morbidities (Popkin, 2006; Hallal *et al.*, 2012). The current food environment consists of foods and beverages that are readily available, inexpensive, energy-dense and high in added sugar and fat (Swinburn *et al.*, 2011). There is an extensive body of literature linking excessive sugar and fat consumption with weight gain, obesity and adverse metabolic health (Malik *et al.*, 2006; Bray and Popkin, 1998; Te Morenga *et al.*, 2013). The sense of taste plays an important role in food intake as the decision to ingest or reject food is governed by the contact between taste stimuli and taste receptors of the gustatory system (Chandrashekar *et al.*, 2006). Recent studies have identified several candidate receptors involved in the chemoreception of FFAs, suggesting a possible role of dietary lipid detection within the gustatory system (Besnard *et al.*, 2016; Galindo *et al.*, 2012). It has previously been suggested that excessive sugar and fat consumption may be driven by lower taste sensitivity (i.e., higher taste thresholds or lower perceived taste intensity) and/or higher hedonic liking for sweet and fat tastes (Deglaire *et al.*, 2015; Jayasinghe *et al.*, 2017; Stewart *et al.*, 2010). However, studies investigating the relationship between different measures of sweet and fat taste perception and measures of obesity are inconclusive and show little agreement.

With regards to sweet taste, a few studies found that overweight or obese individuals have higher sucrose detection thresholds and perceive sweet taste as less intense compared to normal-weight individuals (Ettinger *et al.*, 2012; Sartor *et al.*, 2011). In contrast, a recent study found that obese individuals have lower sucrose thresholds and perceive sweet taste as more intense compared to normal-weight individuals (Hardikar *et al.*, 2017). In regards to fat taste, a few studies have shown that oleic acid hypersensitive individuals (i.e., higher sensitivity) have a lower BMI compared to hyposensitive individuals (Stewart *et al.*, 2010; Stewart *et al.*, 2011a), while others report a positive association between hedonic liking of fat taste and BMI and body fat (Mela and Sacchetti, 1991; Proserpio *et al.*, 2017). However, most studies found no link between any sweet or fat taste perception measurement and BMI or body fat (Pepino *et al.*, 2010; Martinez-Cordero *et al.*, 2015; Low *et al.*, 2016; Chevrot *et al.*, 2014; Stewart and Keast, 2012).

The inconsistent findings between studies may be attributed to the differences in sensory testing methodologies and the experimental design of the studies. For example, the experimental details of the psychophysical techniques used to measure sweet and fat taste

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perception (i.e., type and concentration levels of tastants, method of sample presentation, stopping rule) differ markedly between studies. The experimental setup not only influences sensory data but also makes comparisons of the methods and the results between these studies difficult (Peng *et al.*, 2012; Tucker and Mattes, 2013). Furthermore, taste intensity and hedonic liking measurements obtained from certain rating scales (e.g., VAS or category scales) are not suitable for direct between-group comparisons, as the values or category labels may represent different levels of perceptions for different groups (Bartoshuk *et al.*, 2004b). Therefore, it has been suggested that the gLMS provide a more robust between-group comparison as all perception measurements are made in reference to the ‘strongest imaginable sensation of any kind’ experienced by each individual (Bartoshuk *et al.*, 2005; Bartoshuk *et al.*, 2006). Furthermore, many of the studies were not designed to test differences in sweet and fat taste perception between BMI groups due to small sample sizes and narrow BMI ranges (Low *et al.*, 2016; Ettinger *et al.*, 2012; Sartor *et al.*, 2011; Pepino and Mennella, 2014).

Recent studies have found that taste buds express certain hormones involved in regulating food intake and appetite (e.g., leptin, GLP-1), suggesting that the gustatory system may be intimately linked with the physiological state of the body (Travers and Frank, 2015; Calvo and Egan, 2015). However, the relationship between taste perception and metabolic biomarkers of adiposity has not been extensively investigated (Nakamura *et al.*, 2008; Cruickshanks *et al.*, 2009).

The present study had three main aims. Firstly, to explore differences in sweet taste and fat (creaminess) perception between ethnic groups with known differences in metabolic disease and obesity risk profiles (NZE, Māori, Pacific). Secondly, to explore whether sweet taste and fat (creaminess) perception differ between body composition groups based on BMI and body fat. Thirdly, to assess the direct link between sweet taste and fat (creaminess) perception and metabolic and endocrine biomarkers associated with adiposity and appetite. Taste perception was measured by rating the taste intensity and hedonic liking of sweet taste and creaminess on a gLMS. Body composition groups consisted of standard BMI cut-offs (normal-weight, overweight, obese) and two groups of over and under 35% body fat (Grundy, 2004; World Health Organisation, 2000). Several metabolic and endocrine biomarkers associated with appetite regulation, glucose homeostasis, lipid profile and inflammation were chosen to explore links with sweet taste and fat (creaminess) perception.

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## 4.3 Methods

### 4.3.1 Study design and participants

The data used in the present study are part of the women's EXPLORE study, a cross-sectional study designed to investigate the metabolic disease risks and predictive factors associated with different body fat profiles of NZE, Māori and Pacific women. The EXPLORE study protocol was reviewed and approved by the Massey University Human Ethics Committee (Southern A, Application 13/13) and was registered in the Australian New Zealand Clinical Trials Registry as ACTRN12613000714785. Informed written consent was obtained from all participants prior to participation.

The in-depth details of the EXPLORE study are published elsewhere (Kruger *et al.*, 2015), but the main study details are as follows. A total of 408 post-menarche and pre-menopausal NZE ( $n=233$ ), Māori ( $n=84$ ) and Pacific ( $n=91$ ) women between the ages of 16–45 years were recruited for the study. Māori and Pacific ethnic groups were defined by self-identification and having at least one parent from the same ethnic group. Women were excluded if pregnant or breastfeeding, have irregular menstrual cycles, diagnosed with any chronic illness, allergic to dairy products or currently taking medication that may influence taste perception or saliva production (Steinbach *et al.*, 2009; Redda and Allis, 2006). Participants were recruited from the wider Auckland area through advertisements (i.e., newspapers, magazines, websites), posters and flyers at various venues, social media, e-mail lists and community groups. The recruitment methods for Māori and Pacific ethnic groups were adapted to be culturally appropriate, as advised by the Māori and Pacific advisers of the EXPLORE study.

### 4.3.2 Study procedure

The EXPLORE study was conducted in two phases. Phase 1 of the study involved screening participants for their eligibility and phase 2 involved all data collection. During phase 1 of the study, participants who expressed interest completed a screening questionnaire which assessed their initial eligibility in terms of age, ethnic group and health. Women who fit the initial eligibility criteria were then invited to participate in screening at the Human Nutrition Research Unit at Massey University in Auckland or at an off-site location convenient to the participants. At this screening visit, height using a portable stadiometer, and body weight and total body fat using the bioelectrical impedance analysis machine (Biospace, Inbody 230, Cerritos, California, USA) were obtained. Participant's eligibility was assessed using their BMI and body fat as

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required by the EXPLORE study design (Kruger *et al.*, 2015). Women who met eligibility were invited to participate in phase 2 of the study.

All phase 2 data collection were conducted at the Human Nutrition Research Unit and at the Sensory Research Facilities at Massey University in Auckland. All testing appointments were organised between 7–9.30 a.m. to standardise hunger and diurnal hormonal fluctuations (Gavrila *et al.*, 2003). Participants were tested following an overnight fast and were asked to refrain from brushing their teeth and performing any physical activity an hour prior to testing. Furthermore, phase 2 appointments were scheduled during the first 14 days of the menstrual cycle in order to standardise potential effects of menstrual cycle hormones on taste perception or energy intake (Davidsen *et al.*, 2007) and to ensure participants were not pregnant at the time of data collection. For the purpose of the present study, only methods associated with measurements of sweet taste and fat (creaminess) perception, body composition and metabolic biomarkers will be discussed.

### **4.3.3 Measurements of sweet taste and fat (creaminess) perception**

Sweet taste perception was measured by rating the sweet taste intensity and sweet hedonic liking of five sucrose samples prepared in distilled water; 20 mM (0.6% w/v), 90 mM (3% w/v), 180 mM (6% w/v), 360 mM (12% w/v) and 720 mM (24% w/v) (Holt *et al.*, 2000). In the experimental study in Chapter 3, we found clear links between perceived sweet taste intensity and sweet hedonic liking and various dietary intake measurements. Therefore, in the EXPLORE study we only assessed suprathreshold measurements of taste. Accordingly, sucrose was used as the sweet tastant as it can produce a strong sweet sensation when used at high concentrations and is a type of sugar that is more relevant to the foods people consume (Moskowitz, 1970; New Zealand Nutrition Foundation, 2014). Fat taste perception was measured by rating the creaminess intensity and creaminess hedonic liking of five milk samples varying in fat percentage; 0.1, 1.5, 3.3, 18.5 and 36.9% (Salbe *et al.*, 2004). All sucrose samples were prepared on the day of testing and presented at room temperature (Low *et al.*, 2016), while milk samples, also prepared on the day of testing were refrigerated and presented at 4° C in accordance with previously published protocols (Hayes and Duffy, 2007). Sensory testing was conducted under red light in individual sensory booths set at room temperature (20° C). Participants were instructed to take the whole sample (10 ml) into their mouth, swirl it around for a few seconds, swallow the sample and rinse their mouth with distilled water (Mahar and Duizer, 2007; Holt *et al.*, 2000). Previous studies conducted in our laboratory indicated that participants were uncomfortable with expectorating the tastant samples. Therefore, to maintain

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compliance with the sensory methodology all participants were asked to swallow the tastant samples similar to protocols used by previous publications (Mahar and Duizer, 2007; Holt *et al.*, 2000). All five samples were presented in a random order starting with the sucrose samples followed by the milk samples. Nose clips were not worn during any of the taste measurements to emulate sensory perceptions experienced during normal eating circumstances.

Perceived intensity of each tastant sample was rated on a 100 mm gLMS, ranging from no sensation (0 mm) to strongest imaginable sensation (100 mm) with verbal descriptors assigned to different levels of intensities (Green *et al.*, 1996). Hedonic liking was rated on a hedonic gLMS, ranging from dislike extremely (-100 mm) to like extremely (+100 mm) with neutral (0 mm) in the middle (Lim *et al.*, 2009). Participants were instructed on how to use both rating scales according to the protocol outlined by Green *et al.* 1996. Participants were encouraged to mark anywhere on the scale as they deemed appropriate for their taste experience. For example, if a sample was perceived between moderate and strong sensation, participants were asked to mark closer to the verbal descriptor that more closely represented the sensation.

#### **4.3.4 Body composition measurements**

All anthropometric measurements were obtained using the International Society for the Advancement of Kinanthropometry (ISAK) protocol (Marfell-Jones *et al.*, 2006). Participant's height was measured using a Harpenden stadiometer and recorded to the nearest 0.1 cm. Waist circumference (WC) and hip circumference (HC) were measured using Lufkin steel tapes according to ISAK protocols (Marfell-Jones *et al.*, 2006). Waist to hip ratios (WHR) were calculated from measured variables. Total body fat was measured by air displacement plethysmography using the thoracic gas volume method and in recommended clothing (i.e., swimwear and swim cap) (BodPod, 2007A, Life Measurement Inc., Concord, California, USA) (Wingfield *et al.*, 2014). Participant's weight was measured on the electronic scale attached to the air displacement plethysmography device. Height and weight was used to calculate BMI and stratified according to BMI groups of; normal-weight (18.5–24.9 kg/m<sup>2</sup>), overweight (25–29.9 kg/m<sup>2</sup>) and obese ( $\geq 30$  kg/m<sup>2</sup>) (World Health Organisation, 2000). As no standardised guidelines for body fat cut-offs exist, over and under 35% was considered as high and normal body fat respectively (Oliveros *et al.*, 2014; Grundy, 2004).

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### 4.3.5 Analysis of metabolic and endocrine biomarkers

Fasting blood samples were collected into ethylenediaminetetraacetic acid (EDTA) and serum vacutainers by qualified phlebotomists between 7–9.30 a.m. prior to taste perception measurements. An aliquot of whole EDTA blood was frozen at -80° C for HbA1c analysis. Within an hour of collection, the vacutainers were centrifuged at 3500rpm at 4° C for 15 minutes and aliquots were frozen at -80° C until analysis. Metabolic biomarkers associated with appetite regulation (leptin, ghrelin), glucose homeostasis (glucose, insulin, HbA1c), lipid profile (cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglycerides) and inflammation (C-reactive protein (CRP), interleukin-6 (IL-6), interleukin-10 (IL-10), tumour necrosis factor-alpha (TNF- $\alpha$ )) were chosen to explore the links with sweet taste and fat (creaminess) perception.

Serum insulin was measured by the automated analyser ADVIA centaur system using a chemiluminescent two-site sandwich immunoassay method (Siemens Healthcare Diagnostics, catalogue # 02230141-128434) (Schiaffini *et al.*, 2010). Standard automated laboratory procedures of the dimension vista system (Siemens Healthcare Diagnostics) were used to measure serum cholesterol (cholesterol esterase and cholesterol oxidase method, catalogue # K1027) (Dirinck *et al.*, 2015), CRP (catalogue # K7032), HDL-C (cholesterol oxidase and cholesterol esterase method, catalogue # K3048) (Tosi *et al.*, 2016), glucose (hexokinase and glucose-6-phosphate dehydrogenase method, catalogue # K1039) (Olson *et al.*, 2012) and triglycerides (lipase and glycerol kinase method, catalogue # K2069) (Dirinck *et al.*, 2015). Serum LDL-C levels were calculated from measured variables. The HbA1c levels were measured on frozen EDTA whole blood using the high performance liquid chromatography method (Biorad Variant instrumentation, USA) (Kopprasch *et al.*, 2009). Milliplex immunoassay kits (Millipore Corporation, Billerica, USA) were used to simultaneously measure plasma levels of IL-6, IL-10, TNF- $\alpha$  (catalogue # HSTCMAG-28SK), ghrelin and leptin (catalogue # HMHEMAG-34K) as previously described (Gabel *et al.*, 2016). The assay was performed according to the manufacturer's instructions. The plates were read on Bioplex 200 multiplex system (Bio-Rad Laboratories, Hercules, California, USA) and data were analysed using Bioplex manager software (V.6.0, Bio-Rad Laboratories, Hercules, California, USA).

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## **4.3.6 Data handling and statistical analysis**

### **4.3.6.1 Incomplete data**

A total of 408 participants completed phase 2 of the study. Following the blood analysis two participants were excluded due to their HbA1c levels being higher than 50 mmol/mol (New Zealand Guidelines Group., 2012). A further eight participants had no taste data and were excluded from the analysis. Of the remaining 398 participants, complete sweet taste and fat (creaminess) perception data were available for 393 and 387 participants respectively.

### **4.3.6.2 Statistical analysis**

All data were analysed using the SPSS software version 24 (IBM corporation, New York, USA). All continuous variables were tested for normality using Kolmogorov-Smirnov test together with analysing histograms and normal Q-Q plots. Non-normal data were log transformed and tested again for normality. Log transformed data were used for CRP, IL-6, IL-10 and leptin. Repeated measures design was used to assess within-subject differences in sweet taste and creaminess intensity and hedonic liking in response to the varying levels of sucrose and fat concentrations. These analyses were conducted using multilevel linear models with each participant as a random intercept, ethnic group as the between-subject variable and controlling for age and randomisation of sensory samples. The differences in body composition and metabolic biomarkers between body composition groups were tested using analysis of covariance (covariates: ethnic group, age) together with post hoc tests to identify where the differences lay. The Bonferroni correction was applied to reduce the likelihood of type 1 errors. Analysis of covariance was used to test whether sweet taste and creaminess perception (intensity and hedonic liking) were different between ethnic groups and body composition groups using age and ethnic group as covariates. Relationships between continuous variables were tested using Pearson's correlation coefficient for parametric data or Spearman's correlation coefficient for non-parametric data. Correlation coefficient values were used to determine the strength of the relationship by the criteria  $\pm 0.1$  = weak,  $\pm 0.3$  = moderate and  $\pm 0.7$  = strong (Linneman, 2011). Continuous variables are reported as mean  $\pm$  standard error of the mean (SEM) and categorical data are reported as *n* (%). A  $p < 0.05$  was considered statistically significant.

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## 4.4 Results

### 4.4.1 Participant characteristics

Characteristics of participants by ethnic group are reported in Table 4.1. We found that Pacific women were younger than NZE women ( $p<0.001$ ). Furthermore, Māori and Pacific women had higher body weight and BMI than NZE women ( $p<0.001$ ), while Pacific women had higher total body fat %, WC, HC and WHR than NZE and Māori women ( $p<0.005$ ).

**Table 4.1 Characteristics of all participants and each ethnic group**

	All participants	NZE	Māori	Pacific
<i>n</i>	398	232	80	86
Age (years)	30.9±0.4	32.1±0.5	29.9±1.0	28.6±1.0 <sup>bbb</sup>
Height (cm)	166.9±0.3	167.1±0.4	166.1±0.7	167.2±0.6
Body weight (kg)	75.9±0.9	70.2±0.9	77.0±1.8 <sup>aaa</sup>	90.0±2.1 <sup>bbb ccc</sup>
BMI (kg/m <sup>2</sup> )	27.2±0.3	25.2±0.3	27.9±0.7 <sup>aaa</sup>	32.2±0.7 <sup>bbb ccc</sup>
Total body fat (%)	34.2±0.4	32.6±0.5	34.2±0.9	38.2±0.8 <sup>bbb ccc</sup>
WC (cm)	82.3±0.7	78.3±0.7	83.8±1.4 <sup>aa</sup>	91.8±1.5 <sup>bbb ccc</sup>
HC (cm)	107.2±0.6	104.1±0.6	107.3±1.3	115.4±1.3 <sup>bbb ccc</sup>
WHR	0.77±0.003	0.75±0.004	0.78±0.006 <sup>aaa</sup>	0.79±0.007 <sup>bbb</sup>

NZE: New Zealand European, BMI: body mass index, WC: waist circumference, HC: hip circumference, WHR: waist to hip ratio. Data reported as mean ± SEM. Differences between ethnic groups were tested by one-way analysis of variance and post hoc test (with a Bonferroni correction).

<sup>a</sup> Significantly different between NZE and Māori women, <sup>a</sup>  $p<0.05$ , <sup>aa</sup>  $p<0.01$ , <sup>aaa</sup>  $p<0.001$ .

<sup>b</sup> Significantly different between NZE and Pacific women, <sup>b</sup>  $p<0.05$ , <sup>bb</sup>  $p<0.01$ , <sup>bbb</sup>  $p<0.001$ .

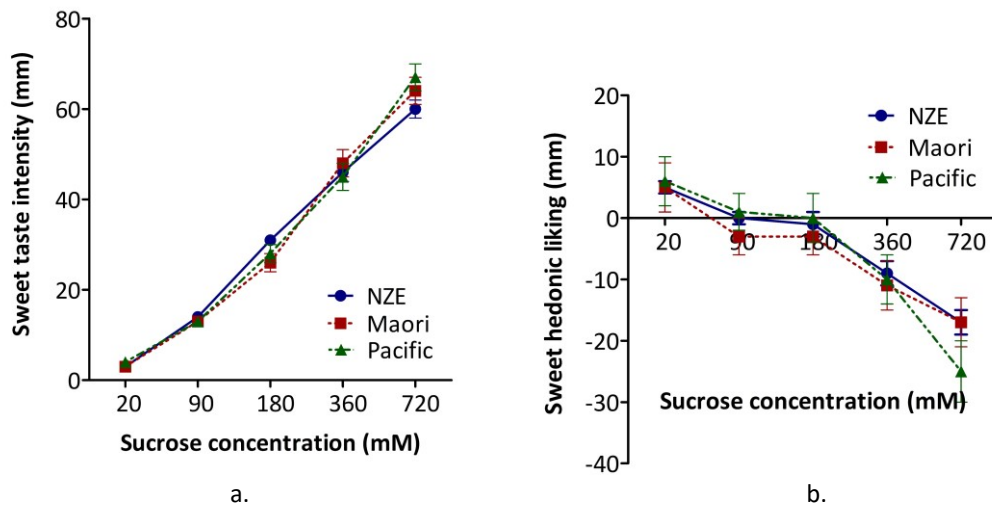
<sup>c</sup> Significantly different between Māori and Pacific women, <sup>c</sup>  $p<0.05$ , <sup>cc</sup>  $p<0.01$ , <sup>ccc</sup>  $p<0.001$ .

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## 4.4.2 Taste perception differences within and between ethnic groups

### 4.4.2.1 Sweet taste intensity and sweet hedonic liking

Figure 4.1 shows the mean sweet taste intensity and sweet hedonic liking ratings of each sucrose concentration for each ethnic group separately. Repeated measures analysis showed that all women across all ethnic groups had a significant main effect of sucrose concentration on sweet taste intensity ratings ( $p < 0.001$ ) and had significantly higher sweet taste intensity ratings with increasing sucrose concentrations ( $p < 0.001$ , Figure 4.1a). Furthermore, sweet taste intensity ratings of individual sucrose concentrations were not significantly different between ethnic groups ( $p > 0.05$ ).



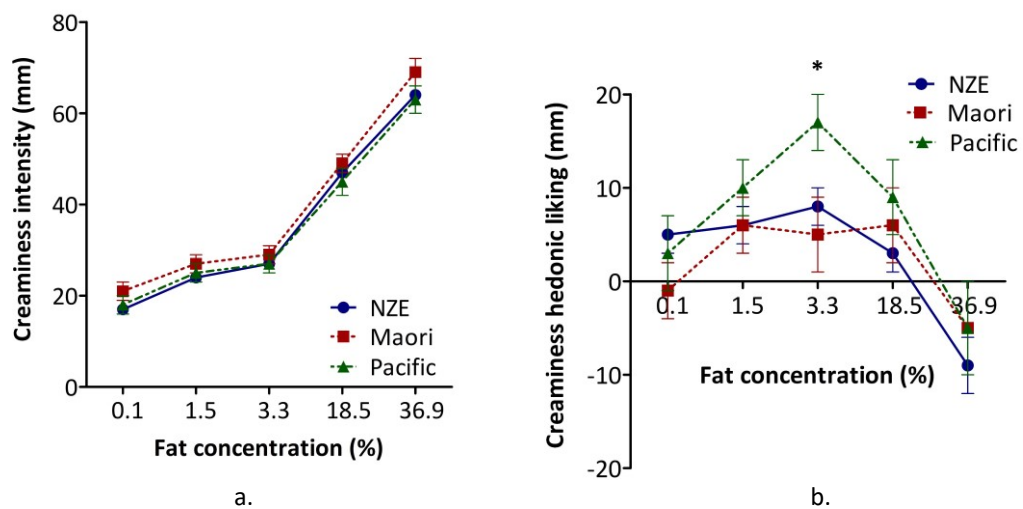
**Figure 4.1 Sweet taste perception ratings of each ethnic group**

The mean sweet taste intensity (a) and sweet hedonic liking (b) ratings of ethnic groups obtained using the general labelled magnitude scale. NZE: New Zealand European. Data reported as mean  $\pm$  SEM.

There was a significant main effect of sucrose concentration on sweet hedonic liking ratings for all ethnic groups ( $p < 0.001$ , Figure 4.1b). Repeated measures analysis revealed that NZE and Pacific women disliked the sweetness of sucrose concentrations above 180 mM ( $p = 0.003$ ). Māori women showed no significant difference in sweet hedonic liking between any consecutive sucrose concentrations (all  $p > 0.05$ ). Furthermore, sweet hedonic liking ratings of individual sucrose concentrations were not significantly different between ethnic groups ( $p > 0.05$ , Figure 4.1b).

#### 4.4.2.2 Creaminess intensity and creaminess hedonic liking

Figure 4.2 shows the mean creaminess intensity and creaminess hedonic liking ratings of the milk samples with varying levels of fat concentrations for each ethnic group separately. There was a significant main effect of fat concentration on creaminess intensity ratings across all ethnic groups ( $p < 0.001$ , Figure 4.2a). Repeated measures analysis showed that all ethnic groups had higher creaminess ratings with increasing fat concentrations, apart from the creaminess of 1.5% and 3.3% samples which was perceived the same. Creaminess intensity ratings of individual fat concentrations were not significantly different between ethnic groups ( $p > 0.05$ , Figure 4.2a).



**Figure 4.2 Creaminess perception ratings of each ethnic group**

The mean creaminess intensity (a) and creaminess hedonic liking (b) ratings of ethnic groups obtained using the general labelled magnitude scale. NZE: New Zealand European. Data reported as mean  $\pm$  SEM. \* Significantly different between Māori and Pacific participants as tested by analysis of covariance,  $p = 0.01$ .

All ethnic groups showed a significant main effect of fat concentration on creaminess hedonic liking ratings ( $p = 0.006$ , Figure 4.2b). Repeated measures analysis showed that all ethnic groups had a significant decrease in creaminess hedonic liking from 3.3% to 36.9% ( $p < 0.01$ ). Interestingly, we observed that Pacific women had a bi-phasic response where there was an initial rise in creaminess hedonic liking from 0.1% to 3.3% and then a clear decrease with further increases in fat concentrations ( $p < 0.001$ ). The only difference in creaminess hedonic liking between ethnic groups was observed at 3.3%, where Pacific women had a higher hedonic liking rating than Māori women ( $p = 0.01$ , Figure 4.2b).

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### 4.4.3 Characteristics of body composition groups

As there were no robust differences in sweet taste and creaminess perception (intensity and hedonic liking) between ethnic groups, the pooled sample of all ethnic groups was used to categorise BMI and body fat groups. Participant characteristics of BMI and body fat groups are reported in Table 4.2.

Obese women were older than normal-weight women ( $p=0.006$ ) and had higher body weight, BMI, total body fat %, WC, HC and WHR than normal-weight and overweight women ( $p<0.005$ ). Furthermore, obese women had higher plasma levels of leptin, insulin, triglyceride and CRP and lower plasma levels of ghrelin and HDL-C compared to normal-weight and overweight women (all  $p<0.001$ ). Women in the high body fat group were older and had higher body weight, BMI, total body fat %, WC, HC and WHR than women in the normal body fat group (all  $p<0.005$ ). Furthermore, the high body fat group had higher plasma levels of leptin, glucose, insulin, triglyceride, CRP and TNF-alpha and lower plasma levels of ghrelin and HDL-C compared to the normal body fat group (all  $p<0.005$ ) (Table 4.2).

**Table 4.2 Characteristics of body composition groups**

	Normal healthy range	BMI groups			Body fat groups	
		Normal-weight (18.5–24.9 kg/m <sup>2</sup> )	Overweight (25–29.9 kg/m <sup>2</sup> )	Obese (≥30 kg/m <sup>2</sup> )	Normal (<35% BF)	High (>35% BF)
<i>n</i>		179	115	104	225	173
Ethnic group ( <i>n</i> (%))						
NZE		140 (78%)	60 (52%)	32 (31%)	155 (69%)	77 (45%)
Māori		28 (16%)	29 (25%)	23 (22%)	40 (18%)	40 (23%)
Pacific		11 (6%)	26 (23%)	49 (47%)	30 (13%)	56 (32%)
Age (years)		27.9±1.0	30.3±0.8	32.1±0.9 <sup>bb</sup>	28.8±0.7	31.6±0.7 <sup>dd</sup>
Body weight (kg)		64.1±1.2	74.7±1.0 <sup>aaa</sup>	98.3±1.0 <sup>bbb ccc</sup>	68.6±1.1	89.0±1.0 <sup>ddd</sup>
BMI (kg/m <sup>2</sup> )		22.8±0.4	27.2±0.3 <sup>aaa</sup>	35.6±0.3 <sup>bbb ccc</sup>	24.5±0.4	32.2±0.3 <sup>ddd</sup>
Total body fat (%)		28.1±0.6	34.6±0.5 <sup>aaa</sup>	43.5±0.5 <sup>bbb ccc</sup>	28.8±0.4	41.6±0.4 <sup>ddd</sup>
WC (cm)	<80 cm	72.8±0.8	82.4±0.7 <sup>aaa</sup>	99.0±0.7 <sup>bbb ccc</sup>	76.3±0.8	92.8±0.7 <sup>ddd</sup>
HC (cm)		99.4±0.8	106.4±0.7 <sup>aaa</sup>	121.3±0.7 <sup>bbb ccc</sup>	102.0±0.7	115.6±0.7 <sup>ddd</sup>
WHR	<0.8	0.73±0.01	0.77±0.01 <sup>aaa</sup>	0.82±0.01 <sup>bbb ccc</sup>	0.75±0.01	0.80±0.01 <sup>ddd</sup>
Leptin (ng/mL)		4.32±0.001	8.79±0.001 <sup>aaa</sup>	16.60±0.001 <sup>bbb ccc</sup>	5.24±0.001	13.84±0.001 <sup>ddd</sup>
Ghrelin (pg/mL)		56.58±4.75	52.21±3.74	30.57±3.88 <sup>bbb ccc</sup>	54.43±3.27	36.27±299 <sup>ddd</sup>
Glucose (mmol/L)	3.5–5.4 mmol/L	4.57±0.05	4.71±0.04	4.83±0.04 <sup>bbb</sup>	4.62±0.03	4.80±0.03 <sup>ddd</sup>
Insulin (mU/mL)	3–25 mU/mL	8.82±0.78	11.86±0.64 <sup>aa</sup>	19.50±0.66 <sup>bbb ccc</sup>	9.55±0.57	17.27±0.53 <sup>ddd</sup>
HbA1c (mmol/mol)	<40 mmol/mol	29.10±0.42	29.10±0.34	29.77±0.36	29.00±0.29	29.49±0.27

	Normal healthy range	BMI groups			Body fat groups	
		Normal-weight (18.5–24.9 kg/m <sup>2</sup> )	Overweight (25–29.9 kg/m <sup>2</sup> )	Obese (≥30 kg/m <sup>2</sup> )	Normal (<35% BF)	High (>35% BF)
Cholesterol (mmol/L)	<5 mmol/L	4.53±0.11	4.50±0.09	4.54±0.09	4.54±0.08	4.48±0.07
HDL-C (mmol/L)	>1 mmol/L	1.64±0.05	1.51±0.04	1.33±0.04 <sup>bbb cc</sup>	1.61±0.03	1.40±0.03 <sup>ddd</sup>
LDL-C (mmol/L)	0–3.4 mmol/L	2.52±0.10	2.58±0.08	2.65±0.08	2.55±0.07	2.58±0.06
Triglyceride (mmol/L)	<2 mmol/L	0.81±0.08	0.97±0.06	1.23±0.07 <sup>bbb ccc</sup>	0.87±0.06	1.10±0.05 <sup>ddd</sup>
CRP (mg/L)	0–5 mg/L	3.21±1.05	3.39±1.04	4.09±1.04 <sup>bbb cc</sup>	3.22±1.03	3.79±1.03 <sup>ddd</sup>
IL-6 (pg/mL)		2.19±1.09	1.93±1.07	2.26±1.07	1.94±1.06	2.20±1.05
IL-10 (pg/mL)		12.33±1.13	12.13±1.10	11.43±1.11	11.56±1.09	11.32±1.08
TNF-α (pg/mL)		7.03±0.30	7.35±0.24	7.33±0.25	6.81±0.21	7.46±0.19 <sup>d</sup>

BMI: body mass index, BF: body fat, NZE: New Zealand European, WC: waist circumference, HC: hip circumference, WHR: waist to hip ratio, HbA1c: glycosylated haemoglobin, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, CRP: C-reactive protein, IL-6: interleukin-6, IL-10: interleukin 10, TNF-α: tumour necrosis factor-alpha. Continuous variables are reported as estimated marginal means ± SEM and categorical data as *n* (%). Differences between BMI and body fat groups tested by analysis of covariance (covariates; ethnic group, age) and post hoc test (with a Bonferroni correction).

<sup>a</sup> Significantly different between normal-weight and overweight groups, <sup>a</sup> *p*<0.05, <sup>aa</sup> *p*<0.01, <sup>aaa</sup> *p*<0.001.

<sup>b</sup> Significantly different between normal-weight and obese groups, <sup>b</sup> *p*<0.05, <sup>bb</sup> *p*<0.01, <sup>bbb</sup> *p*<0.001.

<sup>c</sup> Significantly different between overweight and obese groups, <sup>c</sup> *p*<0.05, <sup>cc</sup> *p*<0.01, <sup>ccc</sup> *p*<0.001.

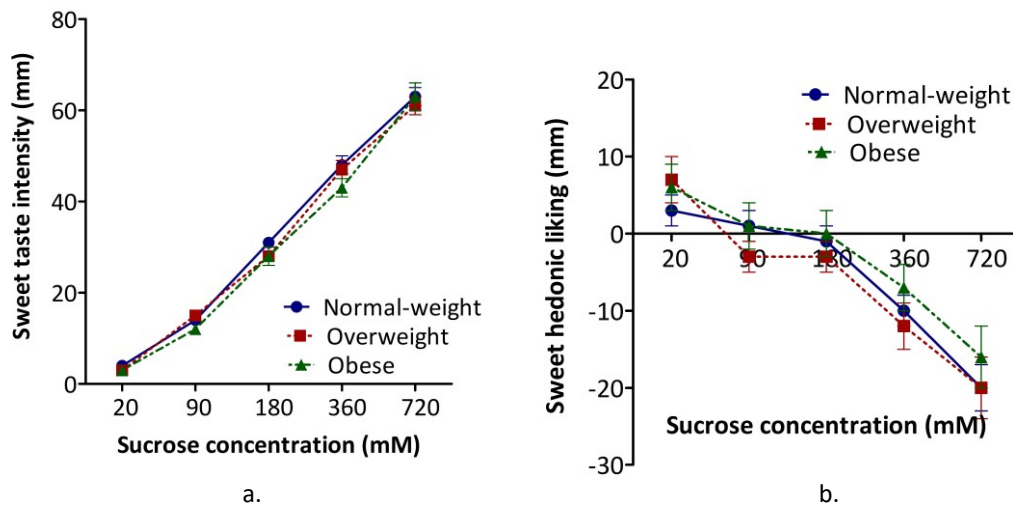
<sup>d</sup> Significantly different between body fat groups, <sup>d</sup> *p*<0.05, <sup>dd</sup> *p*<0.01, <sup>ddd</sup> *p*<0.001.

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#### 4.4.4 Taste perception differences between body composition groups

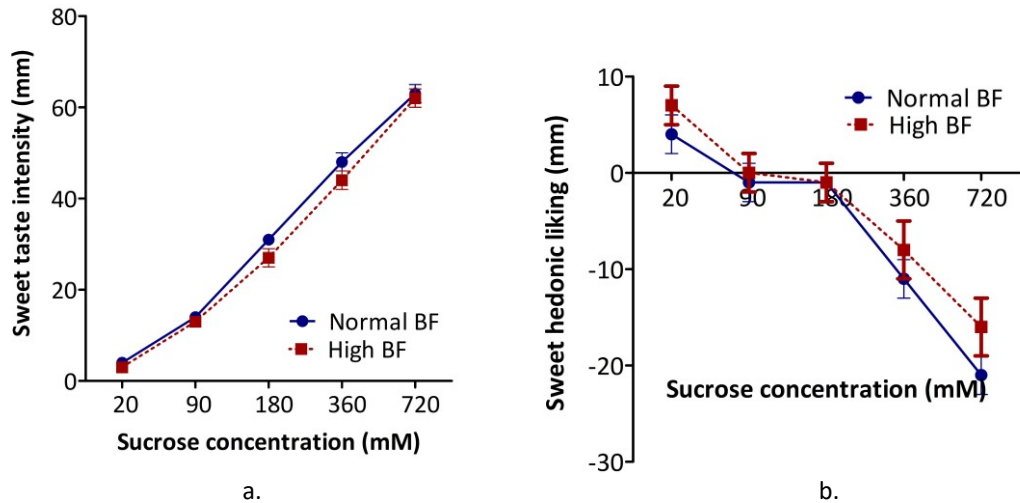
##### 4.4.4.1 Sweet taste intensity and hedonic liking

Sweet taste perception (taste intensity and hedonic liking) ratings of BMI and body fat groups are shown in Figure 4.3 and Figure 4.4 respectively. There was a significant main effect of sucrose concentration on sweet taste intensity and sweet hedonic liking ratings across all BMI and body fat groups ( $p < 0.001$ ). We also observed that all BMI and body fat groups had progressively higher sweet taste intensity ratings with increasing sucrose concentrations (Figure 4.3a and Figure 4.4a) and that all groups showed a clear dislike of sweetness above 180 mM sucrose (Figure 4.3b and Figure 4.4b  $p < 0.001$ ). Furthermore, sweet taste intensity and sweet hedonic liking ratings of individual sucrose concentrations were not significantly different between BMI or body fat groups ( $p > 0.05$ ).



**Figure 4.3 Sweet taste perception ratings of BMI groups**

The above graphs represent the sweet taste intensity (a) and sweet hedonic liking (b) ratings of BMI groups obtained using the general labelled magnitude scale. BMI: body mass index. Normal-weight (18.5–24.9 kg/m<sup>2</sup>)  $n=179$ , overweight (25–29.9 kg/m<sup>2</sup>)  $n=115$ , obese ( $\geq 30$  kg/m<sup>2</sup>)  $n=104$  (World Health Organisation, 2000). Data reported as mean  $\pm$  SEM.

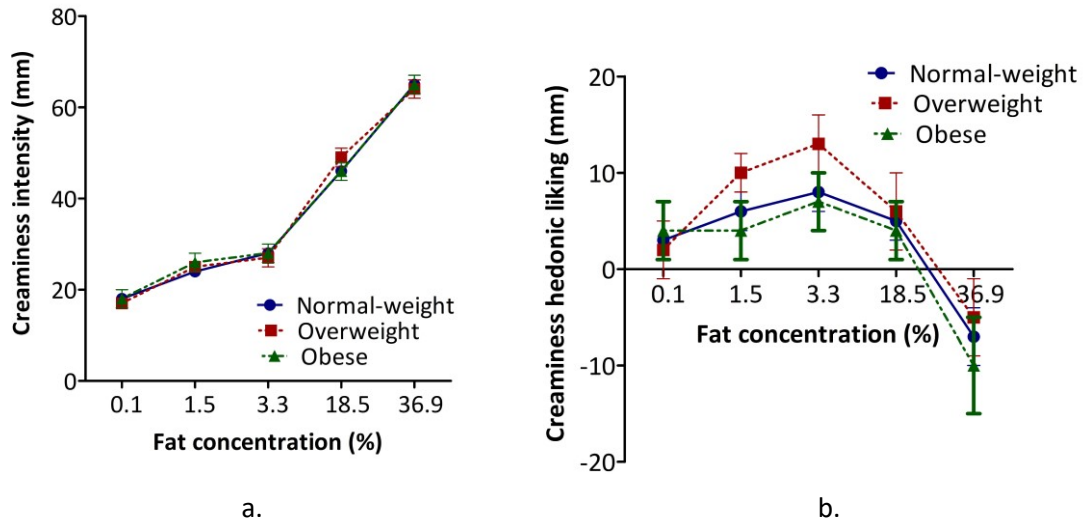


**Figure 4.4 Sweet taste perception ratings of body fat groups**

The above graphs represent the sweet taste intensity (a) and sweet hedonic liking (b) ratings of body fat groups obtained using the general labelled magnitude scale. BF: body fat. Normal BF (<35%)  $n=225$  and high BF (>35%)  $n=173$  (Grundy, 2004). Data reported as mean  $\pm$  SEM.

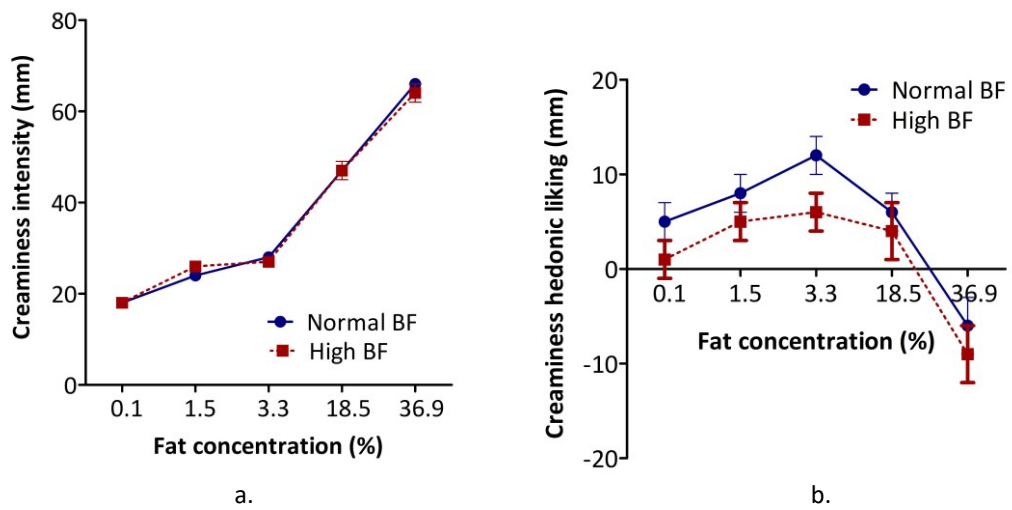
#### 4.4.4.2 Creaminess intensity and hedonic liking

Creaminess perception ratings (creaminess intensity and hedonic liking) of each BMI and body fat group are shown in Figure 4.5 and Figure 4.6 respectively. There was a significant main effect of fat concentration on creaminess intensity and creaminess hedonic liking across all BMI and body fat groups ( $p<0.001$ ). All body composition groups had higher creaminess intensity ratings with increasing fat concentrations apart from 1.5% and 3.3%, which was perceived the same (Figure 4.5a and Figure 4.6a). All BMI and body fat groups showed no difference in creaminess liking between any consecutive fat concentration apart from a significant decrease in liking from 18.5 to 36.9% (Figure 4.5b and Figure 4.6b,  $p<0.001$ ). Furthermore, creaminess intensity and creaminess hedonic liking ratings of individual fat concentrations were not significantly different between BMI or body fat groups ( $p>0.05$ ).



**Figure 4.5 Creaminess perception ratings of BMI groups**

The above graphs represent the creaminess intensity (a) and creaminess hedonic liking (b) ratings of BMI groups obtained using the general labelled magnitude scale. BMI: body mass index. Normal-weight (18.5–24.9 kg/m<sup>2</sup>) *n*=179, overweight (25–29.9 kg/m<sup>2</sup>) *n*=115, obese ( $\geq 30$  kg/m<sup>2</sup>) *n*=104 (World Health Organisation, 2000). Data reported as mean  $\pm$  SEM.



**Figure 4.6 Creaminess perception ratings of body fat groups**

The above graphs represent the creaminess intensity (a) and creaminess hedonic liking (b) ratings of body fat groups obtained using the general labelled magnitude scale. BF: body fat. Normal BF (<35%) *n*=225 and high BF (>35%) *n*=173 (Grundy, 2004). Data reported as mean  $\pm$  SEM.

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#### **4.4.5 Relationship between taste perception and metabolic biomarkers**

To assess the relationship between taste perception and metabolic and endocrine biomarkers, we conducted correlational analysis between sweet taste and creaminess intensity and hedonic liking ratings of the five samples and plasma levels of metabolic biomarkers. We observed a weak negative correlation between triglyceride levels and sweet taste intensity of 180 mM and 360 mM sucrose ( $r=0.01-0.02$ ,  $p<0.05$ ) and a weak positive correlation between LDL-C levels and sweet hedonic liking of 180 mM, 360 mM and 720 mM sucrose ( $r=0.01-0.02$ ,  $p<0.05$ ). There were no other significant correlations between sweet taste and fat (creaminess) perception and metabolic biomarkers.

### **4.5 Discussion**

The aims of the present study were to investigate differences in sweet taste and fat (creaminess) perception between ethnic groups with known differences in metabolic disease and obesity risk and across different body composition groups. Further, to assess the direct link between sweet taste and fat (creaminess) perception and metabolic and endocrine biomarkers of adiposity and appetite. The results of the present study showed no robust differences in sweet taste intensity or sweet hedonic liking between ethnic groups. Furthermore, apart from Pacific women showing a bi-phasic response for creaminess hedonic liking (i.e., an initial increase in liking then a decrease) and liking the 3.3% milk sample more than Māori women, no other differences in fat taste perception between ethnic groups were observed. With regards to body composition groups, we observed no differences in sweet taste and fat (creaminess) perception between BMI or body fat groups. Similarly, there were no strong direct correlations between any sweet taste and fat (creaminess) perception measurement and metabolic biomarkers. Overall, these results suggest that there is no robust link between sweet taste and fat (creaminess) perception, ethnic group, body composition or metabolic biomarkers in this group of healthy adult women.

#### **4.5.1 Sweet taste and fat (creaminess) perception differences between ethnic groups**

The findings of the present study showed that all women across all three ethnic groups could differentiate between progressively increasing sucrose concentrations (i.e., increasing sweet taste intensity ratings with increasing sucrose concentrations). Furthermore, women from all three ethnic groups perceived the same level of sweet taste intensity and had similar sweet hedonic liking ratings. With regards to creaminess perception, all ethnic groups showed increasing creaminess intensity ratings with increasing fat concentrations apart from the

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creaminess of 1.5% and 3.3% which was perceived the same. It is likely that the fat content of 1.5% and 3.3% milk samples were too similar and did not produce a creaminess sensation strong enough to be differentiated orally. We observed a bi-phasic creaminess hedonic liking response in Pacific women, where there was an increase in liking from 0.1% to 3.3% and a decrease in liking for fat concentrations above 3.3%. This bi-phasic creaminess hedonic liking response may suggest that the creaminess hedonic liking pattern of Pacific women is different to other ethnic groups. With the exception of Pacific women liking the creaminess of 3.3% milk sample more than Māori women, we observed no other differences in creaminess intensity or creaminess hedonic liking ratings between ethnic groups.

Differences in sweet and fat taste perception between different ethnic groups have only been explored in a handful of studies (Salbe *et al.*, 2004; Holt *et al.*, 2000). One study reported that obesity prone Pima Indians perceived sucrose solutions as sweeter than Caucasian individuals, but creaminess intensity was not significantly different between the ethnic groups (Salbe *et al.*, 2004). Another study reported that African American and Hispanic individuals perceive sucrose solutions as more sweet than non-Hispanic White individuals (Williams *et al.*, 2016). These studies indicate that there are taste perception differences between ethnic groups with different obesity risks.

To the best of our knowledge, our study is the first to investigate differences in sweet taste and fat (creaminess) perception within and between ethnic groups in New Zealand. As reported in the latest New Zealand Health Survey, Māori and Pacific women have a higher risk of metabolic disease and obesity (52% and 73% obese respectively), while NZE women have a moderate risk of metabolic disease and obesity (32% obese) (Ministry of Health, 2017). Differences in metabolic disease and obesity risk between different ethnic groups may be attributed to differences in diet and/or physical activity patterns or socio-economic inequalities (Metcalf *et al.*, 2008; Ministry of Health, 2015a; Ministry of Health, 2006). Therefore, understanding taste perception differences between ethnic groups, at least in part, may explain differences in dietary habits and intake (Metcalf *et al.*, 2014; Metcalf *et al.*, 2008). However, we observed no robust differences in sweet taste and fat (creaminess) perception between NZE, Māori and Pacific women of the present study. This suggests that the differences in obesity risk observed between the ethnic groups of the present study may be associated with other determinants of health (e.g., social and economic factors) and not directly linked with sweet or fat taste perception (Utter *et al.*, 2018; Reremoana *et al.*, 2015). For example, according to the New Zealand Health Survey 2016/17, adults living in the most deprived areas were 1.5 times as likely to be obese as adults living in the least deprived areas (Ministry of Health, 2017). Furthermore,

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the differences in obesity risk are most likely contributed by the food choices driven by socio-economic factors such as poverty and income (Glanz *et al.*, 1998; Newton *et al.*, 2017). Therefore, future studies should investigate the influence of broader determinants of health on food choice and intake, especially when investigating population groups with known differences in obesity and metabolic disease risk.

#### **4.5.2 Characteristics of body composition groups**

Although women in the obese and high body fat groups were older than the normal-weight and normal body fat groups respectively, it is important to note that the mean age of all body composition groups (~30 years) indicate a relatively young study population. We observed significantly higher body weight, BMI, total body fat %, WC, HC and WHR in the obese and high body fat groups compared to normal-weight and normal body fat groups respectively, indicating well-defined body composition groups with different levels of adiposity.

In terms of metabolic biomarkers, plasma leptin levels reflected adiposity (Liuzzi *et al.*, 1999) and were highest in the obese and high body fat groups compared to the other groups. As previously shown to be characteristic in response to increased adiposity, we observed a state of hyper-insulin secretion and lower circulating ghrelin levels in the obese and high body fat groups in comparison to the normal-weight and normal body fat groups respectively (Carlson *et al.*, 2009; Saltiel, 2012; Polonsky *et al.*, 1988). Although a slightly higher level of fasting glucose was found in the obese and high body fat groups compared to the other groups, fasting glucose levels of all groups were within the normal healthy range (i.e., <5.4 mmol/L) (New Zealand Guidelines Group., 2012). Plasma HbA1c levels (a biomarker used to measure long-term blood glucose levels) of all body composition groups further confirmed that the study participants had normal glucose metabolism (New Zealand Guidelines Group., 2012). Furthermore, lipid and inflammatory markers were within the normal healthy ranges. Overall, these findings show clearly that the study population of the present study were generally young, healthy and showed no pathophysiology, nonetheless showed significant differences in body composition and metabolic and endocrine biomarkers that paralleled the differences in adiposity between BMI and body fat groups.

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### 4.5.3 Relationship between sweet taste and fat (creaminess) perception and body composition

Our results showed that all BMI and body fat groups could differentiate between increasing sucrose concentrations and perceive the same level of sweet taste intensity at each sucrose concentration. These findings are in line with studies that reported no associations between sweet taste intensity and BMI or body fat (Salbe *et al.*, 2004; Low *et al.*, 2016; Pepino *et al.*, 2010). In contrast to our findings, others report that overweight/obese individuals find sucrose solutions to be less intense (Ettinger *et al.*, 2012; Sartor *et al.*, 2011) or more intense (Hardikar *et al.*, 2017) compared to normal-weight individuals. Consistent with finding of others, our study found no significant differences in sweet hedonic liking ratings between BMI or body fat groups (Ettinger *et al.*, 2012; Sartor *et al.*, 2011).

We found no significant link between body composition and creaminess intensity or creaminess hedonic liking similar to the findings of others (Salbe *et al.*, 2004; Pepino and Mennella, 2014; Tucker *et al.*, 2017). These findings contrast with other studies that report a negative relationship between linoleic acid intensity ratings and BMI ( $r=-0.2$ ) (Martínez-Ruiz *et al.*, 2014) and to others who showed a positive relationship between fat preference and BMI and body fat ( $r=0.46$ ) (Mela and Sacchetti, 1991; Proserpio *et al.*, 2017). The inconsistent findings between studies may be related to differences in sweet and fat taste perception measurements (e.g., type of tastant, concentration levels, ratings scales) and to differences in the experimental design and participant characteristics (e.g., sample size, BMI and body fat range, age).

The overall findings of the present study indicate that in this group of healthy participants, there were no robust differences in sweet and fat taste intensity or hedonic liking between the well-defined BMI and body fat groups. Supporting the findings of our study, a recent systematic review concluded that fat taste intensity ratings do not differ among individuals of different weight categories (Tucker *et al.*, 2017). Inter-individual variations in sweet and fat taste intensity and hedonic liking may be an important factor influencing the intakes of sugar- and fat-rich food. However, as body composition is influenced by many other variables including physical activity, dietary intake and lifestyle factors (e.g., smoking), the direct link between taste perception and body composition may be more difficult to isolate.

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#### 4.5.4 Relationship between sweet taste and fat (creaminess) perception and biomarkers

In the present study, apart from a few weak correlations, we found no strong direct associations between sweet taste and fat (creaminess) perception and metabolic and endocrine biomarkers of adiposity and appetite. We believe the lack of associations could be attributed to the relatively young and generally healthy study population.

In the present study, there was a weak negative correlation between fasting triglyceride levels and sweet intensity of 180 mM and 360 mM sucrose ( $r=0.01-0.02$ ). This suggested that women who found these sucrose concentrations as more sweet had lower levels of fasting triglycerides compared to women who found it less sweet. Furthermore, women who had a higher hedonic liking for 180 mM, 360 mM and 720 mM sucrose had higher levels of LDL-C compared to women with a lower sweet hedonic liking ( $r=0.01-0.02$ ). It has been suggested that elevated triglyceride and LDL-C levels are a causal risk factor for inflammation and atherosclerotic cardiovascular disease (Nordestgaard, 2016; Hokanson and Austin, 1996). Our results indicate, although weakly, that either a lower perceived sweet intensity or higher sweet hedonic liking may be linked with higher levels of triglyceride and LDL-C. As discussed in Chapter 3, a lower perceived sweet intensity and a higher sweet hedonic liking is linked with higher intakes of energy and carbohydrate (starch and sugar) (Jayasinghe *et al.*, 2017). Previous research has shown that higher intakes of dietary free-sugar is associated with higher levels of plasma lipids (e.g., triglyceride, total cholesterol, LDL-C) (Te Morenga *et al.*, 2014). Therefore, we could speculate that the link between sweet taste perception and higher levels of triglyceride and LDL-C could be indirectly mediated by higher intakes of sugar (Te Morenga *et al.*, 2014). It is important to note that in our study, triglyceride and LDL-C levels of all body composition groups were within the normal health range (New Zealand Guidelines Group., 2012). We could speculate that stronger correlations between metabolic biomarkers and sweet taste and fat (creaminess) perception measurements may be found in individuals with a more varied dietary intake (i.e., a diet higher in sweet and fat foods).

We observed no significant relationships between sweet taste and fat (creaminess) perception and leptin. Several previous studies have reported a link between sucrose detection thresholds and leptin concentrations (Nakamura *et al.*, 2008; Umabiki *et al.*, 2010). For example, one study reported a positive correlation between sweet taste thresholds and plasma leptin levels in normal-weight individuals; lowest thresholds in the morning (lower leptin levels) and the highest thresholds at night (higher leptin levels) (Nakamura *et al.*, 2008). However, it has been previously discussed that sweet taste thresholds (detection and recognition) are not linked with

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sweet taste intensity or hedonic liking of suprathreshold concentrations (Webb *et al.*, 2015; Jayasinghe *et al.*, 2017). Therefore, although a link between sweet taste thresholds and leptin concentrations in previous studies was observed, we cannot be certain whether a similar relationship between leptin and sweet taste intensity or sweet hedonic liking exists.

#### **4.5.5 Strengths and limitations of the study**

Our study has several strengths. Firstly, as different ethnic groups with known differences in obesity risk were recruited, women were classified by their BMI and body fat content. Secondly, the body composition groups were well-defined in terms of BMI, body fat and differences in endocrine biomarkers, indicating groups with quite distinct adiposity and physiology. Thirdly, the use of the gLMS provided the most robust and valid approach for between-group taste perception comparisons (Bartoshuk *et al.*, 2005). Furthermore, by not using nose-clips during sensory testing we emulated normal eating experiences and allowed cross-modal interactions between taste and olfaction that usually takes place during eating experiences.

Several limitations of the present study should also be addressed. Due to significant challenges encountered during participant recruitment, sample sizes of Māori and Pacific women were smaller than NZE women. We believe this reduction in statistical power may have contributed to the lack of taste perception differences observed between ethnic groups. However, data from this study shows clearly that sweet taste and fat (creaminess) perception does not explain differences in obesity risk between the three ethnic groups. One limitation of this study is the lack of data on other determinants of health (e.g., income, index of deprivation, education level) that are known to influence obesity (Ministry of Health, 2015c; Southwick *et al.*, 2012; Reremoana *et al.*, 2015). Therefore, further studies are needed to explore the influence of broader determinants of health on food choice, obesity and metabolic health within each ethnic group separately. Furthermore, participants were relatively young (~30 years), were in good general health and showed no pathophysiology as indicated by the metabolic biomarkers. We believe these characteristics of the study participants may have contributed to the lack of associations between taste, body composition and metabolic biomarkers. Past studies have shown differences in taste perception in individuals with altered metabolic disease statuses such as diabetes (Wasalathanthri *et al.*, 2014; Perros *et al.*, 1996) or following gastric bypass surgery (Nance *et al.*, 2018; Pepino *et al.*, 2014). Future studies in women and men with different metabolic health statuses should be conducted to support or challenge the current findings. The tastants used in the present study (i.e., sucrose dissolved in water and milk solutions) may not have accurately reflected normal eating experiences, thereby influencing participant's taste

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rating responses. For example, sweet taste and fat (creaminess) perception of liquid tastants is not comparable to the sensory properties exerted from solid foods or beverages. In addition, we observed high variances for sweet and creaminess hedonic liking measurements which may have contributed to the lack of differences between ethnic and body composition groups. Rating sweet and fat taste perception of five samples are commonly used in similar research settings (Holt *et al.*, 2000). By reducing the number of tastant samples to four, we may improve the variances of taste measurements so the true biological differences in taste perceptions between groups can be identified. Furthermore, with regards to creaminess perception, it is possible that other sensory attributes such as mouthfeel and olfaction played a role, thereby influencing the creaminess intensity and hedonic liking ratings.

## 4.6 Conclusions

The present study showed that women from different ethnic groups with known differences in metabolic disease and obesity risk and different body composition profiles perceived the same level of sweet and fat taste intensity and had similar sweet and fat hedonic liking. This suggests that the differences in obesity risk and body composition profiles are influenced by other determinants of health and not directly linked with sweet or fat taste perception. Future studies should investigate how socio-economic determinants influence food choice and intake in order to understand risk factors associated with obesity, particularly in high risk population groups. We also found no robust direct relationships between sweet taste and fat (creaminess) perception and metabolic and endocrine biomarkers of adiposity and appetite were found. We believe these results are attributed to the characteristics of the study participants who had significantly different body composition but no pronounced differences in metabolic and endocrine biomarkers, indicating a general healthy study population. Given the multi-modal processes involved in creaminess taste perception, future work should assess gustation, olfaction and textural properties separately, using standardised methods of testing and real food systems to provide better comparisons with real-life eating experiences (e.g., popcorn with varying levels of butter). As discussed above, measures should be taken to reduce the variance in sensory data by reducing the number tastant concentration levels and including real food systems, so the true physiological differences in taste perception between groups can be ascertained. Furthermore, larger trials within each ethnic group with a larger range of BMI and body fat should be conducted to support or challenge the current findings.

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## Chapter 5

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### ***The Relationship Between Dietary Patterns, Body Composition and Metabolic Biomarkers***

#### **5.1 Abstract**

The combinations of food consumed together (dietary patterns) may have a greater influence on health than nutrients or food groups independently. The objective of our study was to assess the links between dietary patterns, body composition, macronutrient intakes and metabolic and endocrine biomarkers in women with different metabolic disease and obesity risk. A total of 408 NZE, Māori and Pacific women aged 16–45 years were recruited. Dietary intake was assessed using a 220-item FFQ. Several parameters of body composition and metabolic and endocrine biomarkers were measured. Dietary patterns were extracted by principal component analysis (PCA) and dietary pattern scores were categorised into tertiles to assess links with other measured parameters. Four dietary patterns were identified; refined and processed, sweet and savoury snacking, fruit and vegetable, and fats and meat. Compared to women with lower scores, women with higher scores for the ‘refined and processed’ pattern were younger, had higher BMI and total body fat ( $p<0.001$ ) and had higher intakes of total energy, carbohydrate, starch and total sugar ( $p<0.05$ ). Furthermore, women with higher scores for the ‘refined and processed’ pattern had higher plasma insulin and leptin levels ( $p<0.001$ ) and lower ghrelin and HDL-C levels ( $p<0.05$ ) than women with lower scores. No differences in body composition (after controlling for energy intake) or metabolic biomarkers were found between tertiles of other dietary patterns. Our findings highlight the diet-induced metabolic dysregulation associated with the ‘refined and processed’ dietary pattern characterised by the differences in body composition and metabolic and endocrine biomarkers across the dietary pattern tertiles. These findings also highlight that dietary pattern analysis is a useful tool to investigate how diet relates to obesity and metabolic health.

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## 5.2 Introduction

The prevalence of overweight and obesity continues to increase worldwide (Roberto *et al.*, 2015). High BMI is a risk factor for many chronic illnesses, including type 2 diabetes, cardiovascular disease, stroke and certain cancers (The GBD Obesity Collaborators, 2017; Hall *et al.*, 2014). Although the aetiology of obesity is both multi-factorial and complex, obesity is a state of positive energy balance due to higher energy intake (i.e., diet) relative to energy expenditure (i.e., physical activity) (Hall *et al.*, 2011). Therefore, it is important to identify specific dietary determinants that increase the risk of obesity. Traditionally, individual nutrients (e.g., fat, carbohydrate) and food groups (e.g., vegetables, dairy) have been the focus of research exploring the link between diet, obesity and metabolic health (Cox *et al.*, 1999; Beydoun *et al.*, 2008; Jebb, 2007). However, dietary intake is inherently complex as the cumulative effects of various nutrients gained from eating different foods can be either beneficial or harmful to health (Jacobs and Tapsell, 2013). More recently, dietary pattern analysis has emerged as a complementary method of characterising dietary intakes (Appel *et al.*, 1997; Newby *et al.*, 2004; Hu, 2002). Dietary patterns represent a broader picture of the overall diet as it reflects the combinations of foods and nutrients consumed together. Therefore, dietary pattern analysis is considered a useful tool to assess links between diet and nutrition-related health outcomes (Hu, 2002 ; Moeller *et al.*, 2007).

Dietary patterns in a population can be derived a priori or a posteriori using dietary data obtained from food records, FFQs or 24-hour diet recalls (Moeller *et al.*, 2007; Hu, 2002). Dietary patterns derived a priori use pre-defined scientific knowledge such as dietary indices and dietary recommendations, whereas a posteriori methods use data-driven statistical approaches such as factor analysis and cluster analysis (Moeller *et al.*, 2007; Hu, 2002). Principal component analysis, a well-established method of factor analysis, derives dietary patterns based on the inter-correlations between dietary variables (i.e., food groups), thereby simplifying a large number of variables into a smaller set of factors (i.e., dietary patterns). With PCA, the output is a continuous variable where each individual is given a score reflective of their intake for a dietary pattern. Thus, it describes the variation in intake between individuals (i.e., higher scores indicate higher intakes). On the other hand, cluster analysis derives patterns based on the differences in intake between individuals and differentiates individuals into non-overlapping groups of varying dietary patterns. Each individual belongs to one cluster only and clusters can be used as categorical variables for data analysis (Moeller *et al.*, 2007; Hu, 2002).

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Relationships between dietary patterns and metabolic health have been explored by several cross-sectional (Paradis *et al.*, 2009; Suliga *et al.*, 2015) and longitudinal (Newby *et al.*, 2003; Pala *et al.*, 2013) studies. In general, higher intakes of the ‘Western’ or ‘unhealthy’ dietary patterns characterised by refined grains (e.g., white bread, bagels), take-away food, red and processed meats and soft drinks have been associated with higher body weight, BMI, adiposity and waist to hip ratio (Paradis *et al.*, 2009; Bell *et al.*, 2015). On the other hand, following a ‘Prudent’ or ‘healthy’ dietary pattern characterised by fruits, vegetables, wholegrains (e.g., whole wheat, brown rice), eggs, fish and seafood have been associated with a lower BMI and adiposity and a smaller increase in BMI and waist circumference (Newby *et al.*, 2003; Paradis *et al.*, 2009; Suliga *et al.*, 2015).

Despite many studies investigating the associations between dietary patterns and health outcomes, to our knowledge dietary patterns of New Zealand populations have only been explored by a handful of studies (Beck *et al.*, 2017; Wall *et al.*, 2016; Thompson *et al.*, 2010). A recent study using 24-hour diet recall data from a sample of 4657 adults reported that a ‘healthy’ dietary pattern was positively associated with age, female gender and NZE ethnic group and negatively associated with BMI and waist circumference (Beck *et al.*, 2017). On the other hand, a ‘traditional’ dietary pattern was positively associated with male gender, smoking and food insecurity (Beck *et al.*, 2017). However, there is a dearth of studies that explored the links between dietary patterns, metabolic health biomarkers and endocrine regulators. Obesity rates in New Zealand has increased substantially over the past three decades (Ministry of Health, 2017). The latest New Zealand Health Survey reports that Māori and Pacific women have a higher metabolic disease and obesity risk (52% and 73% obese respectively), while NZE women have a moderate metabolic disease and obesity risk (32% obese) (Ministry of Health, 2017). Therefore, it is important to understand whether a link exists between dietary patterns and metabolic health markers in ethnic groups with known differences in metabolic disease and obesity risk.

The main aim of the present study was to explore the links between dietary patterns, body composition, macronutrient intakes and metabolic and endocrine biomarkers in women from three ethnic groups (NZE, Māori and Pacific) with different metabolic disease and obesity risk. The findings of our study will further our understanding about the links between dietary patterns, obesity and metabolic health and help guide future dietary recommendations as diet is a modifiable risk factor for many chronic illnesses.

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## 5.3 Methods

### 5.3.1 Study design

The data used in the present study are part of the women's EXPLORE study, a cross-sectional study designed to investigate the metabolic disease risks associated with different body fat profiles of NZE, Māori and Pacific women. More details of the EXPLORE study can be found elsewhere (Kruger *et al.*, 2015). Ethics approval was obtained from the Massey University Human Ethics Committee (Southern A, Application 13/13). The EXPLORE study was registered in the Australian New Zealand Clinical Trials Registry as ACTRN12613000714785. All participants provided informed written consent prior to participating in the study.

### 5.3.2 Study participants

Post-menarche and pre-menopausal women of NZE ( $n=233$ ), Māori ( $n=84$ ) and Pacific ( $n=91$ ) ethnic groups between the ages of 16–45 years participated in the study. Our power calculation determined that a minimum of 30 participants per body composition group will provide 80% power at a significance level of 0.05 to detect a medium size effect for comparing different body fat profile groups. Māori and Pacific ethnic groups were defined by self-identification and having at least one parent from the same ethnic group (Kruger *et al.*, 2015). Exclusion criteria of the study were women who were pregnant or breastfeeding, have irregular menstrual cycles, diagnosed with any chronic illness, allergic to dairy products or currently on medication that may influence taste perception (Steinbach *et al.*, 2009; Redda and Allis, 2006). Participants were recruited from the Auckland area using various recruitment methods, including advertising in newspapers and magazines, using social media and email lists and through contacts with community groups. Culturally appropriate recruitment methods were used for Māori and Pacific ethnic groups.

### 5.3.3 Study procedure

The study was conducted in two phases; phase 1 (screening) and phase 2 (data collection). Using a screening questionnaire participant's initial eligibility of age, ethnic group and health were determined. Participants who fit the initial eligibility were screened at the Human Nutrition Research Unit at Massey University in Auckland or at an off-site location. At the screening phase participant's height (stadiometer), and weight and body fat % (Bioelectrical impedance analysis, Biospace, Inbody 230, California, USA) were obtained to determine their eligibility as required by the EXPLORE study design (Kruger *et al.*, 2015). Women who met eligibility, participated in

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the phase 2 data collection at the Human Nutrition Research Unit at Massey University in Auckland.

Phase 2 appointments were scheduled during the first 14 days of the menstrual cycle in order to standardise effects of menstrual cycle hormones on food intake (Davidsen *et al.*, 2007) and to be certain that women were not pregnant at the time of data collection. To standardise hunger and diurnal hormonal fluctuations (Gavrila *et al.*, 2003), all testing appointments were organised following an overnight fast between 7–9.30 a.m. For the present study, methods associated with body composition, metabolic biomarkers and dietary pattern analysis will be discussed.

#### **5.3.4 Body composition measurements**

All anthropometric measurements were obtained using the ISAK protocols (Marfell-Jones *et al.*, 2006). Body weight was measured to the nearest 0.1 kg using electronic scales and height was measured with a Harpenden stadiometer to the nearest 0.1 cm. Waist circumference (WC) and HC were measured using Lufkin steel tapes according to the ISAK protocol. Waist to hip ratios (WHR) were calculated from measured variables. Total body fat was measured by full body composition analysis using the air displacement plethysmography device and the thoracic gas volume method (BodPod, 2007A, V4.2+ software, Life Measurement Inc., Concord, California, USA) (Wingfield *et al.*, 2014). Android and gynoid fat were determined from whole body dual-energy X-ray absorptiometry (Hologic QDR Discovery A with APEX v3.2 software, Hologic Inc., Bedford, Massachusetts, USA).

#### **5.3.5 Assessing metabolic and endocrine biomarkers**

Fasting venous blood samples were drawn into EDTA and serum vacutainer tubes by qualified phlebotomists. An aliquot of EDTA whole blood was immediately frozen at -80° C for HbA1c analysis. Within an hour of collection, vacutainer tubes were centrifuged at 3500 rpm at 4° C for 15 minutes and aliquots were frozen at -80° C until required. Blood samples were used to measure metabolic and endocrine biomarkers associated with appetite regulation (leptin, total ghrelin), glucose homeostasis (insulin, glucose, HbA1c), lipid profile (cholesterol, HDL-C, LDL-C, triglyceride) and inflammation (IL-6, IL-10, TNF- $\alpha$ , CRP).

Serum insulin was measured by the automated analyser ADVIA Centaur system using a chemiluminescent two-site sandwich immunoassay method (Siemens Healthcare Diagnostics, catalogue # 02230141-128434) (Schiaffini *et al.*, 2010). Using standard automated laboratory procedures of the Dimension Vista system (Siemens Healthcare Diagnostics), levels of serum

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cholesterol (cholesterol esterase and cholesterol oxidase method, catalogue # K1027) (Dirinck *et al.*, 2015), CRP (catalogue # K7032), HDL-C (cholesterol oxidase and cholesterol esterase method, catalogue # K3048) (Tosi *et al.*, 2016), glucose (hexokinase and glucose-6-phosphate dehydrogenase method, catalogue # K1039) (Olson *et al.*, 2012) and triglyceride (lipase and glycerol kinase method, catalogue # K2069) (Dirinck *et al.*, 2015) were measured. Serum LDL-C levels were calculated using measured variables. The HbA1c levels of EDTA whole blood were measured using the high performance liquid chromatography method (Biorad Variant instrumentation, USA) (Kopprasch *et al.*, 2009). Milliplex immunoassay kits (Millipore Corp., Billerica, Massachusetts, USA) were used to simultaneously measure plasma levels of IL-6, IL-10 and TNF- $\alpha$ , (catalogue # HSTCMAG-28SK), and ghrelin and leptin (catalogue # HMHEMAG-34K) as previously described (Gabel *et al.*, 2016).

### **5.3.6 Food frequency questionnaire**

All participants completed a validated 220-item semi-quantitative FFQ to assess the frequency of foods and beverages consumed over the previous month (Appendix 5) (Beck *et al.*, 2018). The FFQ contained food items organised by common food groups such as dairy, breads and cereals, meat, fish, poultry, fats and oils, fruits and vegetables, sweet and savoury snacks and take-away foods. Standard natural portions (e.g., 1 medium banana, 1 medium potato) or standard weights and volumes (e.g., 1 pottle of yoghurt, 1 meat patty) were used to measure the intakes of the food items. For each food item participants selected the intake frequency that best described their usual intake over the previous month, keeping in mind the standard serving size. The frequency of intake categories were; never, less than once per month, 1–3 times per month, once per week, 2–3 times per week, 4–6 times per week, once a day, 2–3 times per day or more than four times per day. The food items in the FFQ were adapted from the 2008/09 New Zealand Adult Nutrition Survey and included culturally specific foods of Māori and Pacific ethnic groups (e.g., Rēwena bread, pipi) (Ministry of Health, 1997; Ministry of Health, 2011).

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## 5.3.7 Data handling and statistical analysis

### 5.3.7.1 Food frequency questionnaire data analysis

All data obtained from the FFQ were first entered into FoodWorks 7 (FoodWorks professional 2013, Xyris Software, Queensland, Australia) using the New Zealand food composite database to determine energy and macronutrient intakes. Participants whose daily energy intakes were <1000 kcal or >5000 kcal (i.e., 4200–21,000 kJ) were excluded from the analysis (University of Otago and Ministry of Health, 2011; Willett, 2013). Four participants were excluded due to incomplete FFQ data and a further 10 and 14 participants were excluded due to under- and over-reporting respectively. Following blood sample analysis, two participants were excluded due to their HbA1c levels being higher than 50 mmol/mol (New Zealand Guidelines Group., 2012). A final sample size of 378 was used for data analysis.

The frequency of intakes of the individual food items were converted to DFEs calculated by allocating proportional values to the original frequency categories with reference to a base value of 1.0, equivalent to once a day. The scores were calculated as: DFE score of 0—never; 0.008—less than once per month; 0.067—1–3 times per month; 0.14—once per week; 0.36—2–3 times per week; 0.71—4–6 times per week; 1—once a day; 2.5—2–3 times per day; 4—more than four times per day. Based on the nutritional composition, food items of the FFQ were categorised into 57 food groups (Appendix 6), similar to a previous publication (e.g., full-fat milk, sweetened milk products, starchy vegetables, red meats) (Schrijvers *et al.*, 2016). Individual items that solely created a food group (e.g., potatoes, yoghurt) had to be consumed by at least 10% of the study participants. For each participant, a single DFE score for each of the 57 food groups was calculated.

### 5.3.7.2 Dietary pattern extraction

As discussed in Chapter 4, we observed no clear differences in sweet taste and fat (creaminess) perception between the ethnic groups in our study. Therefore, the pooled sample of all three ethnic groups was used for dietary pattern extraction. Additionally, the extracted dietary patterns will be used in Chapter 6 to assess the link with sweet taste and fat (creaminess) perception.

Dietary patterns were extracted employing the exploratory factor analysis method and using each participant's DFE scores for each of the 57 food groups. Factors (i.e., dietary patterns) were extracted using PCA and orthogonal rotation (Hamer *et al.*, 2010). Orthogonal (varimax) rotation was chosen as the factors were not highly correlated with each other. The Kaiser-Meyer-Olin

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measure and Bartlett's test was used to assess the sample adequacy of the group. For the present study, the Kaiser-Meyer-Olin measure of sampling adequacy was 0.7 (>0.5 acceptable) and Bartlett's Test  $p$  value was <0.001 (<0.001 acceptable) (Field, 2009). The number of dietary patterns that best suited the data were determined using scree plots, an eigenvalue cut-off >1 and the factor loadings. The extracted dietary patterns were named based on the nutritional characteristics of the food groups with high factor loadings (>0.3), indicating a large contribution to the pattern. A negative loading (>-0.3) indicated a strong inverse relationship between the food group and dietary pattern.

Inter-item reliability of each dietary pattern was assessed using Cronbach's  $\alpha$  ( $\geq 0.7$  = good, 0.5–0.7 = moderate) (Field, 2009) to ensure that the associated food groups were an appropriate measure of the dietary pattern. A further check was done to assess whether the inter-item reliability of the dietary pattern would increase if any food group was removed. For the present study, the inter-item reliability of the dietary patterns did not improve with the removal of any food group. To assess the link between each dietary pattern and body composition, macronutrient intake and metabolic biomarkers, dietary pattern scores were categorised into tertiles to differentiate women with lower scores (i.e., tertile 1, lower intake) from higher scores (i.e., tertile 3, higher intake).

### **5.3.7.3 Statistical analysis**

All data were analysed using SPSS software version 24 (IBM corporation, New York, USA). Continuous variables were tested for normality using Kolmogorov-Smirnov test together with analysing histograms and normal Q-Q plots. Non-normal data were log transformed and tested for normality. Log transformed data were used for leptin, CRP, IL-6 and IL-10. The differences in body composition, macronutrient intake and metabolic biomarkers between the dietary pattern tertiles were tested using analysis of covariance (covariates: ethnic group, age) together with post hoc tests to identify where the differences lay. The Bonferroni correction was applied to reduce the likelihood of type 1 errors. Interactions between categorical variables were assessed using chi-square test together with standardised residuals to test significance. The level of significance was assessed using the standardised residual values by the criteria;  $\pm 1.96 = p < 0.05$ ;  $\pm 2.58 = p < 0.01$ ;  $\pm 3.29 = p < 0.001$  (Field, 2009). Continuous variables are reported as mean  $\pm$  SEM and categorical data as  $n$  (%). A  $p < 0.05$  was considered statistically significant.

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## 5.4 Results

### 5.4.1 Characteristics of study participants

Characteristics of NZE, Māori and Pacific ethnic groups are reported in Table 5.1. Māori and Pacific women had higher body weight and BMI than NZE women ( $p < 0.001$ ), while Pacific women had higher total body fat %, android fat %, WC, HC and higher circulating levels of leptin than NZE and Māori women ( $p < 0.005$ ). We also observed that plasma Insulin levels were higher in Māori and Pacific women than NZE women ( $p < 0.001$ ). Furthermore, Pacific women had higher plasma levels of glucose, HbA1c and TNF- $\alpha$  and lower levels of ghrelin, cholesterol, HDL-C and LDL-C ( $p < 0.05$ ) than NZE women.

**Table 5.1 Characteristics of study participants by ethnic group**

	All participants	NZE	Māori	Pacific
<i>n</i>	378	225	78	75
Age (years)	31.0±0.4	32.0±0.5	30.1±1.0	29.1±1.1 <sup>b</sup>
Body weight (kg)	75.6±0.9	70.3±1.0	77.6±2.0 <sup>aa</sup>	89.4±2.2 <sup>bbb ccc</sup>
BMI (kg/m <sup>2</sup> )	27.1±0.3	25.2±0.3	28.0±0.7 <sup>aaa</sup>	31.9±0.7 <sup>bbb ccc</sup>
BMI groups ( <i>n</i> (%))				
Normal-weight (18.5–24.9 kg/m <sup>2</sup> )	173(46%)	134(59%)	27(35%)	11(14%)
Overweight (25–29.9 kg/m <sup>2</sup> )	110(29%)	60(27%)	28(36%)	23(31%)
Obese (≥30 kg/m <sup>2</sup> )	95(25%)	31(14%)	23(29%)	41(55%)
Total body fat (%)	34.0±0.4	32.6±0.5	34.5±0.9	37.8±0.9 <sup>bbb c</sup>
Body fat groups ( <i>n</i> (%))				
<35%	217(57%)	151(67%)	38(49%)	28(37%)
>35%	161(43%)	74(33%)	40(51%)	47(63%)
Android fat (%)	33.5±0.4	31.8±0.5	34.5±0.9 <sup>a</sup>	37.8±0.8 <sup>bbb c</sup>
Gynoid fat (%)	37.4±0.3	37.4±0.3	36.6±0.6	38.4±0.6
WC (cm)	82.0±0.7	78.4±0.7	83.6±1.3 <sup>aa</sup>	91.2±1.6 <sup>bbb ccc</sup>
HC (cm)	107.0±0.6	104.1±0.7	107.8±1.3 <sup>a</sup>	114.8±1.4 <sup>bbb ccc</sup>
WHR	0.76±0.01	0.75±0.01	0.77±0.01 <sup>aa</sup>	0.79±0.01 <sup>bb</sup>
Leptin (ng/mL)	7.76±0.001	6.67±0.001	8.28±0.001	11.51±0.001 <sup>bbb c</sup>
Ghrelin (pg/mL)	47.87±2.08	52.62±2.65	44.57±4.51	36.41±4.72 <sup>bb</sup>
Glucose (mmol/L)	4.68±0.02	4.63±0.03	4.71±0.05	4.82±0.05 <sup>bb</sup>
Insulin (mU/mL)	12.09±0.45	9.26±0.53	13.51±0.88 <sup>aaa</sup>	19.21±0.92 <sup>bbb ccc</sup>
HbA1c (mmol/mol)	28.63±0.19	27.58±0.23	29.70±0.38 <sup>aaa</sup>	30.74±0.40 <sup>bbb</sup>
Cholesterol (mmol/L)	4.59±0.05	4.77±0.06	4.42±0.10 <sup>aa</sup>	4.21±0.10 <sup>bbb</sup>
HDL-C (mmol/L)	1.57±0.02	1.66±0.03	1.47±0.05 <sup>aaa</sup>	1.39±0.05 <sup>bbb</sup>
LDL-C (mmol/L)	2.58±0.04	2.69±0.06	2.46±0.09	2.40±0.10 <sup>b</sup>
Triglyceride (mmol/L)	0.96±0.03	0.92±0.05	1.08±0.06	0.95±0.06
CRP (mg/L)	3.55±1.02	3.57±1.03	3.56±1.05	3.37±1.05
IL-6 (pg/mL)	1.95±1.05	1.83±1.05	2.10±1.08	2.25±1.08
IL-10 (pg/mL)	10.72±1.05	9.82±1.07	14.42±1.12 <sup>a</sup>	10.52±1.12
TNF-alpha (pg/mL)	6.79±0.13	6.26±0.16	7.65±0.27 <sup>aaa</sup>	7.49±0.29 <sup>bbb</sup>

NZE: New Zealand European, BMI: body mass index, WC: waist circumference, HC: hip circumference, WHR: waist to hip ratio, HbA1c: glycosylated haemoglobin, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, CRP: C-reactive protein, IL-6: interleukin-6, IL-10: interleukin 10, TNF-alpha: tumour necrosis factor-alpha. BMI and body fat groups were classified according to references (World Health Organisation, 2000) and (Grundy, 2004) respectively. Continuous variables are reported as mean ± SEM and categorical data as *n* (%). Differences between ethnic groups were tested by one-way analysis of variance and post hoc test (with a Bonferroni correction).

<sup>a</sup> Significantly different between NZE and Māori women, <sup>a</sup> *p*<0.05, <sup>aa</sup> *p*<0.01, <sup>aaa</sup> *p*<0.001.

<sup>b</sup> Significantly different between NZE and Pacific women, <sup>b</sup> *p*<0.05, <sup>bb</sup> *p*<0.01, <sup>bbb</sup> *p*<0.001.

<sup>c</sup> Significantly different between Māori and Pacific women, <sup>c</sup> *p*<0.05, <sup>cc</sup> *p*<0.01, <sup>ccc</sup> *p*<0.001.

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## 5.4.2 Dietary patterns

Dietary patterns were extracted using DFE scores of the 57 food groups obtained from the 220-item semi-quantitative FFQ. Using the PCA method and varimax rotation, four distinct dietary patterns were identified. The factor loadings of the food groups contributing to the four dietary patterns are reported in Table 5.2. Higher factor loadings of a given food group indicate greater contribution of that food group to the specific dietary pattern. Dietary patterns were named according to the food groups with the highest positive factor loadings.

Pattern one, named 'refined and processed' pattern explained 9% of the variance in food intake. This pattern was characterised by high factor loadings on crumbed and deep-fried food, fast-food, puddings, fruit drinks, soft drinks and other beverages, sweetened milk products, refined grains, starchy vegetables, white breads and sweetened cereals. The 'refined and processed' pattern was also characterised by negative loadings for water and nuts and seeds. Pattern two labelled 'sweet and savoury snacking' pattern explained 7% of the variance in food intake and was characterised by high loadings on cakes and biscuits, sweet and savoury spreads, sweet and savoury snacks, margarine, peanut butter and peanuts, sauces, whole grain breads, cheese and crackers.

Pattern three explained 5% of the variance in food intake and was named 'fruit and vegetable' pattern. This pattern had high factor loadings on all fruits and vegetables (except potatoes), soy products, legumes, wholegrains and yoghurt. Pattern four, 'fats and meat' pattern explained 4% of the variance in food intake and was characterised by high loadings on fats, all red, white and processed meats, creamy dressings, egg dishes, coconut fats, alcoholic beverages and full-fat milk, and negative loadings on low-fat milk. Together, the four dietary patterns accounted for 25% of the variance in food intake. The inter-item reliability of the dietary patterns indicated good reliability for patterns one, two and three (Cronbach's  $\alpha = 0.7$ ) and moderate reliability for pattern four (Cronbach's  $\alpha = 0.5$ ).

**Table 5.2 Food groups and factor loadings of the four dietary patterns**

Food group	Pattern 1 – refined and processed	Pattern 2 – sweet and savoury snacking	Pattern 3 – fruit and vegetable	Pattern 4 – fats and meat
Crumbed and deep-fried food	0.57	-	-	-
Fast-food	0.57	-	-	-
Puddings	0.54	-	-	-
Fruit drinks, soft drinks and other	0.53	-	-	-
Sweetened milk products	0.52	-	-	-
Refined grains	0.50	-	-	-
White breads	0.42	-	-	-
Sweetened cereals	0.34	-	-	-
Water	-0.47	-	-	-
Nuts and seeds	-0.44	-	-	-
Cake and biscuits	-	0.61	-	-
Sweet spreads	-	0.56	-	-
Sweet snack foods	-	0.55	-	-
Savoury snack foods	0.32	0.52	-	-
Margarine	-	0.49	-	-
Peanut butter and peanuts	-	0.46	-	-
Sauces	-	0.46	-	0.35
Savoury spreads	-	0.45	-	-
Whole grain breads	-	0.44	-	-
Crackers	-	0.42	-	-
Low-fat cheese	-	0.37	-	-
High-fat cheese	-	0.33	-	0.32
Dark yellow vegetables	-	-	0.65	-
Green vegetables	-	-	0.63	0.35
Other non-starchy vegetables	-	-	0.62	-
Other fruit	-	-	0.59	-
Starchy vegetables	0.44	-	0.56	-
Apple, banana, orange	-	-	0.53	-
Soy products	-	-	0.47	-
Legumes	-	-	0.41	-
Wholegrains	-	-	0.34	-
yoghurt	-	-	0.32	-
Tomatoes	-	-	0.31	-
Fats	-	-	-	0.54
Red meats	-	-	-	0.54
Creamy dressings	-	0.46	-	0.54
Processed meats	-	-	-	0.51
White meats	-	-	-	0.46
Low-fat milk	-	-	-	-0.42
Egg and egg dishes	-	-	-	0.38
Coconut fats	-	-	-	0.37
Other alcoholic beverages	-	-	-	0.32
Full-fat milk	-	-	-	0.30
Variance of food intake explained	9	7	5	4
Final Cronbach's alpha	0.7	0.7	0.7	0.5

All 57 food groups were derived from the 220-item semi-quantitative food frequency questionnaire. Dietary patterns were extracted using the principal component analysis method and varimax rotation. Only factor loadings >0.3 are reported.  $n=378$ .

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### 5.4.3 Participant characteristics of dietary pattern tertiles

Participants were categorised into tertiles of dietary pattern scores to differentiate women with lower scores (i.e., tertile 1, lower intake) from higher scores (i.e., tertile 3, higher intake) for each dietary pattern separately. Participant characteristics of all dietary pattern tertiles are reported in Table 5.3.

Women with higher scores for the 'refined and processed' pattern were younger, had higher body weight, BMI, total body fat %, android fat %, WC, HC and WHR than women with lower scores, even after controlling for energy intake (all  $p < 0.001$ ). Furthermore, there were significantly more Māori (51%,  $p < 0.05$ ) and Pacific (68%,  $p < 0.001$ ) women and significantly less NZE women (16%,  $p < 0.001$ ) in tertile 3 compared to tertile 1 of the 'refined and processed' pattern.

Women with higher scores for the 'sweet and savoury snacking' pattern had higher body weight, total body fat %, WC and HC than women with lower scores (all  $p < 0.05$ ). However, these differences were no longer significant after controlling for energy intake. There were significantly more NZE women (40%,  $p < 0.01$ ) and significantly less Māori women (15%,  $p < 0.001$ ) in tertile 3 compared to tertile 1 of the 'sweet and savoury snacking' pattern.

There were no significant differences in age, ethnic group or body composition measurements between tertiles of the 'fruit and vegetable' pattern, even after controlling for energy intake (all  $p > 0.05$ ). Women with higher scores for the 'fats and meat' pattern had higher body weight, BMI, android fat %, WC and HC compared to women with lower scores ( $p < 0.005$ ). However, these differences were no longer evident after controlling for energy intake. Furthermore, there were significantly fewer NZE women (27%,  $p < 0.01$ ) and significantly more Māori women (47%,  $p < 0.01$ ) in tertile 3 compared to tertile 1 of the 'fats and meat' pattern.

**Table 5.3 Participant characteristics of dietary pattern tertiles**

	Refined and processed pattern			Sweet and savoury snacking pattern			Fruit and vegetable pattern			Fats and meat pattern		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
Age (years)	34.4 ±1.3	32.4 ±1.0	27.6 ±0.7 <sup>bbb ccc</sup>	29.6 ±0.8	29.6 ±0.9	32.2 ±1.1	31.2 ±0.8	31.0 ±0.9	28.8 ±0.9	29.7 ±1.0	30.5 ±0.9	30.4 ±0.8
Ethnic group ( <i>n</i> (%))												
NZE	101 (45%)	89 (39%)	35 (16%) <sup>bbb c</sup>	54 (24%)	82 (36%)	89 (40%) <sup>bb</sup>	62 (28%)	83 (37%)	80 (36%)	88 (39%)	76 (34%)	61 (27%) <sup>bb c</sup>
Māori	18 (23%)	20 (26%)	40 (51%) <sup>b</sup>	42 (54%)	24 (31%)	12 (15%) <sup>bbb</sup>	33 (42%)	26 (33%)	19 (24%)	16 (21%)	25 (32%)	37 (47%) <sup>bb</sup>
Pacific	7 (9%)	17 (23%)	51 (68%) <sup>bbb c</sup>	30 (40%)	20 (27%)	25 (33%)	31 (41%)	17 (23%)	27 (36%)	22 (29%)	25 (33%)	28 (37%)
Body weight (kg)	72.0 ±2.4	79.7 ±1.8 <sup>a</sup>	82.6 ±1.5 <sup>bbb</sup>	77.5 ±1.5	78.6 ±1.7	84.0 ±1.9 <sup>b</sup>	78.6 ±1.5	78.4 ±1.8	79.9 ±1.7	75.4 ±1.8	78.7 ±1.6	82.7 ±1.5 <sup>bb</sup>
BMI (kg/m <sup>2</sup> )	25.9 ±0.8	28.3 ±0.6	29.9 ±0.5 <sup>bbb</sup>	28.1 ±0.5	28.1 ±0.6	30.1 ±0.7	28.2 ±0.5	28.2 ±0.6	28.5 ±0.6	27.1 ±0.6	28.4 ±0.6	29.6 ±0.5 <sup>bb</sup>
Total body fat (%)	31.9 ±1.2	35.3 ±0.9	37.3 ±0.7 <sup>bbb</sup>	34.0 ±0.7	34.8 ±0.8	37.3 ±1.0 <sup>b</sup>	34.8 ±0.8	35.5 ±0.9	34.5 ±0.9	33.9 ±0.9	34.4 ±0.8	36.6 ±0.7
Android fat (%)	32.0 ±1.1	35.3 ±0.9	36.4 ±0.7 <sup>bb</sup>	34.4 ±0.7	34.5 ±0.8	36.0 ±0.9	34.6 ±0.7	35.2 ±0.8	34.6 ±0.8	33.1 ±0.9	34.9 ±0.8	35.9 ±0.7 <sup>b</sup>
Gynoid fat (%)	36.1 ±0.7	38.1 ±0.6	38.2 ±0.5	36.9 ±0.5	37.4 ±0.5	38.6 ±0.6	37.8 ±0.5	37.5 ±0.6	37.1 ±0.5	36.8 ±0.6	37.5 ±0.5	37.9 ±0.5

	Refined and processed pattern			Sweet and savoury snacking pattern			Fruit and vegetable pattern			Fats and meat pattern		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
WC (cm)	79.0 ±1.7	84.7 ±1.3 <sup>a</sup>	87.6 ±1.0 <sup>bbb</sup>	83.3 ±1.1	84.3 ±1.2	87.8 ±1.4 <sup>b</sup>	84.1 ±1.1	84.5 ±1.3	84.4 ±1.3	82.1 ±1.3	84.1 ±1.2	87.2 ±1.1 <sup>bb</sup>
HC (cm)	104.6 ±1.6	109.5 ±1.2 <sup>a</sup>	111.3 ±1.0 <sup>bb</sup>	108.1 ±1.0	107.7 ±1.1	112.8 ±1.3 <sup>bc</sup>	108.9 ±1.0	108.8 ±1.2	108.7 ±1.1	106.8 ±1.2	108.3 ±1.1	111.3 ±1.0 <sup>b</sup>
WHR	0.75 ±0.01	0.77 ±0.01 <sup>a</sup>	0.79 ±0.01 <sup>bc</sup>	0.77 ±0.01	0.78 ±0.01	0.78 ±0.01	0.77 ±0.01	0.77 ±0.01	0.77 ±0.01	0.77 ±0.01	0.77 ±0.01	0.78 ±0.01

T1, T2, T3: dietary pattern tertiles 1,2 and 3. NZE: New Zealand European, BMI: body mass index, WC: waist circumference, HC: hip circumference, WHR: waist to hip ratio.  $n=126$  in each tertile. Continuous variables are reported as estimated marginal means  $\pm$  SEM and categorical variables as  $n$  (%). Differences between tertiles for continuous variables were tested by analysis of covariance (covariates: ethnic group, age) and post hoc test (with a Bonferroni correction). Differences between categorical variables were tested by chi-square test and standardised residuals.

<sup>a</sup> Significantly different between tertiles 1 and 2, <sup>a</sup>  $p<0.05$ , <sup>aa</sup>  $p<0.01$ , <sup>aaa</sup>  $p<0.001$ .

<sup>b</sup> Significantly different between tertiles 1 and 3, <sup>b</sup>  $p<0.05$ , <sup>bb</sup>  $p<0.01$ , <sup>bbb</sup>  $p<0.001$ .

<sup>c</sup> Significantly different between tertiles 2 and 3, <sup>c</sup>  $p<0.05$ , <sup>cc</sup>  $p<0.01$ , <sup>ccc</sup>  $p<0.001$ .

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#### 5.4.4 Energy and macronutrient intakes of dietary pattern tertiles

Energy and macronutrient intakes of the dietary pattern tertiles are reported in Table 5.4. All dietary pattern tertiles were within the AMDR for protein intake (15–25%) (National Health and Medical Research Council, 2006). Interestingly, all dietary pattern tertiles were closer to or slightly above the upper limit of the AMDR for fat intake (20–35%), and were closer to or slightly lower than the lower limit of the AMDR for carbohydrate intake (45–65%) (National Health and Medical Research Council, 2006).

Women with higher scores (tertile 3) for the ‘refined and processed’ pattern had significantly higher intakes of total energy ( $p<0.001$ ), percentage carbohydrate ( $p<0.001$ ), starch ( $p<0.002$ ), total sugar ( $p<0.05$ ) and sucrose ( $p=0.002$ ) and significantly lower intakes of percentage fat and protein ( $p<0.004$ ) compared to women with lower scores (tertile 1). Women with higher scores for the ‘sweet and savoury snacking’ pattern had significantly higher intakes of total energy and percentage starch ( $p<0.001$ ) and significantly lower intakes of percentage protein ( $p<0.001$ ), glucose ( $p=0.001$ ) and fructose ( $p=0.01$ ) than women with lower scores.

Women with higher scores for the ‘fruits and vegetable pattern’ had significantly higher intakes of total energy ( $p<0.001$ ), percentage protein ( $p<0.05$ ), carbohydrate ( $p<0.05$ ), total sugar ( $p<0.001$ ), glucose ( $p<0.001$ ) and fructose ( $p<0.001$ ) and a significantly lower intake of percentage fat ( $p<0.001$ ) compared to women with lower scores. Women with higher scores for the ‘fats and meat’ pattern had significantly higher intakes of total energy ( $p<0.001$ ), percentage protein and fat ( $p<0.001$ ) and significantly lower intakes of percentage carbohydrate, starch, total sugar and fructose (all  $p<0.005$ ) than women with lower scores.

**Table 5.4 Energy and macronutrient intakes of dietary pattern tertiles**

	Refined and processed pattern			Sweet and savoury snacking pattern			Fruit and vegetable pattern			Fats and meat pattern		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
Total energy (MJ)	8.5 ±0.5	8.5 ±0.4	11.8 ±0.3 <sup>bbb ccc</sup>	7.7 ±0.3	10.6 ±0.3 <sup>aaa</sup>	12.7 ±0.4 <sup>bbb ccc</sup>	8.8 ±0.3	10.2 ±0.4 <sup>a</sup>	11.6 ±0.4 <sup>bbb c</sup>	8.9 ±0.4	8.7 ±0.3	12.1 ±0.3 <sup>bbb ccc</sup>
Protein (g)	96.5 ±5.8	93.5 ±4.4	121.2 ±3.5 <sup>bbb ccc</sup>	90.7 ±3.4	113.1 ±4.0 <sup>aaa</sup>	123.7 ±4.5 <sup>bbb</sup>	90.9 ±3.5	108.7 ±4.1 <sup>aa</sup>	127.1 ±4.0 <sup>bbb cc</sup>	87.7 ±4.0	94.0 ±3.5	133.5 ±3.3 <sup>bbb ccc</sup>
Protein (%) (15–25%) <sup>d</sup>	19.5 ±0.5	19.0 ±0.4	17.5 ±0.3 <sup>bbc</sup>	20.0 ±0.3	18.0 ±0.4 <sup>aaa</sup>	16.6 ±0.4 <sup>bbb cc</sup>	17.8 ±0.3	18.4 ±0.4	19.0 ±0.4 <sup>b</sup>	16.7 ±0.4	18.7 ±0.3 <sup>aaa</sup>	19.3 ±0.3 <sup>bbb</sup>
Fat (g)	90.1 ±5.8	82.2 ±4.4	108.7 ±3.5 <sup>b ccc</sup>	72.7 ±3.1	102.3 ±3.6 <sup>aaa</sup>	124.8 ±4.1 <sup>bbb ccc</sup>	89.3 ±3.6	100.3 ±4.2	105.7 ±4.1 <sup>bb</sup>	80.0 ±3.9	81.0 ±3.4	124.4 ±3.2 <sup>bbb ccc</sup>
Fat (%) (20–35%) <sup>d</sup>	38.7 ±1.0	35.2 ±0.7 <sup>a</sup>	34.3 ±0.6 <sup>bbb</sup>	34.9 ±0.6	35.7 ±0.7	36.8 ±0.8	37.3 ±0.6	36.1 ±0.7	33.3 ±0.7 <sup>bbb cc</sup>	33.0 ±0.7	34.8 ±0.6	38.6 ±0.6 <sup>bbb ccc</sup>
Carbohydrate (g)	181.9 ±14.9	210.1 ±11.2	316.1 ±9.0 <sup>bbb ccc</sup>	190.5 ±8.7	266.4 ±10.2 <sup>aaa</sup>	322.2 ±11.6 <sup>bbb ccc</sup>	217.1 ±9.6	253.3 ±11.3	300.2 ±11.1 <sup>bbb cc</sup>	245.8 ±12.2	226.7 ±10.8	283.2 ±10.0 <sup>bbb</sup>
Carbohydrate (%) (45–65%) <sup>d</sup>	36.7 ±1.0	42.1 ±0.8 <sup>aaa</sup>	45.5 ±0.6 <sup>bbb cc</sup>	41.5 ±0.7	42.4 ±0.8	43.0 ±0.9	41.4 ±0.7	41.7 ±0.8	44.0 ±0.8 <sup>b</sup>	46.8 ±0.8	42.9 ±0.7 <sup>aaa</sup>	38.3 ±0.6 <sup>bbb ccc</sup>
Starch (%)	16.5 ±0.9	20.2 ±0.7 <sup>aa</sup>	22.4 ±0.5 <sup>bbb c</sup>	19.4 ±0.5	20.6 ±0.6	22.8 ±0.7 <sup>bbb</sup>	21.7 ±0.6	20.0 ±0.7	20.1 ±0.6	23.1 ±0.7	21.1 ±0.6	18.6 ±0.5 <sup>bbb cc</sup>
Total sugar (%)	20.2 ±0.9	22.0 ±0.7	23.1 ±0.6 <sup>b</sup>	22.1 ±0.6	21.8 ±0.7	20.1 ±0.8	19.7 ±0.6	21.7 ±0.7	23.9 ±0.6 <sup>bbb</sup>	23.7 ±0.7	21.8 ±0.6	19.7 ±0.6 <sup>bbb c</sup>

	Refined and processed pattern			Sweet and savoury snacking pattern			Fruit and vegetable pattern			Fats and meat pattern		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
Glucose (%)	4.4 ±0.3	4.6 ±0.2	4.6 ±0.2	4.7 ±0.2	4.6 ±0.2	3.8 ±0.2 <sup>bbb c</sup>	3.7 ±0.2	4.5 ±0.1 <sup>aa</sup>	5.2 ±0.2 <sup>bbb c</sup>	4.7 ±0.2	4.4 ±0.1	4.2 ±0.2
Fructose (%)	4.4 ±0.3	4.8 ±0.2	5.1 ±0.2	5.1 ±0.2	4.6 ±0.2	4.1 ±0.3 <sup>b</sup>	3.7 ±0.2	4.7 ±0.2 <sup>aa</sup>	5.7 ±0.2 <sup>bbb cc</sup>	5.3 ±0.2	4.6 ±0.2	4.2 ±0.2 <sup>bb</sup>
Sucrose (%)	7.6 ±0.5	8.4 ±0.4	9.6 ±0.3 <sup>bb c</sup>	8.2 ±0.3	8.7 ±0.4	8.7 ±0.4	8.4 ±0.3	8.6 ±0.4	8.6 ±0.4	9.0 ±0.4	8.7 ±0.3	8.0 ±0.3
Lactose (%)	3.3 ±0.4	3.8 ±0.3	3.4 ±0.3	3.8 ±0.3	3.5 ±0.3	3.0 ±0.3	3.3 ±0.3	3.5 ±0.3	4.0 ±0.3	4.1 ±0.3	3.6 ±0.3	2.8 ±0.3 <sup>bb</sup>
Maltose (%)	0.43 ±0.03	0.41 ±0.03	0.53 ±0.02 <sup>cc</sup>	0.40 ±0.02	0.48 ±0.02	0.57 ±0.03 <sup>bbb c</sup>	0.54 ±0.02	0.46 ±0.02	0.43 ±0.02 <sup>bb</sup>	0.55 ±0.03	0.48 ±0.02	0.41 ±0.02 <sup>bbb c</sup>

T1, T2, T3: dietary pattern tertiles 1, 2 and 3. Percentage energy from macronutrients calculated as a % of total energy intake.  $n=126$  in each tertile. Data reported as estimated marginal means  $\pm$  SEM. Differences between tertiles tested by analysis of covariance (covariates: ethnic group, age) and post hoc test (with a Bonferroni correction).

<sup>a</sup> Significantly different between tertiles 1 and 2, <sup>a</sup>  $p<0.05$ , <sup>aa</sup>  $p<0.01$ , <sup>aaa</sup>  $p<0.001$ .

<sup>b</sup> Significantly different between tertiles 1 and 3, <sup>b</sup>  $p<0.05$ , <sup>bb</sup>  $p<0.01$ , <sup>bbb</sup>  $p<0.001$ .

<sup>c</sup> Significantly different between tertiles 2 and 3, <sup>c</sup>  $p<0.05$ , <sup>cc</sup>  $p<0.01$ , <sup>ccc</sup>  $p<0.001$ .

<sup>d</sup> Acceptable macronutrient distribution range (National Health and Medical Research Council, 2006).

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#### **5.4.5 Metabolic and endocrine biomarkers of dietary pattern tertiles**

Measurements of metabolic and endocrine biomarkers of all dietary pattern tertiles are presented in Table 5.5. It is important to note that all metabolic biomarkers were within the normal healthy ranges for all dietary pattern tertiles (New Zealand Guidelines Group., 2012), which indicates a generally healthy study population. However, women with higher scores (tertile 3) for the 'refined and processed' pattern had significantly higher levels of insulin and leptin ( $p<0.001$ ) and lower levels of ghrelin ( $p=0.03$ ) and HDL-C ( $p<0.001$ ) compared to women with lower scores (tertile 1). Furthermore, women with higher scores for the 'sweet and savoury snacking' pattern had a significantly higher level of leptin ( $p=0.007$ ) compared to women with lower scores. We observed no significant differences in metabolic biomarkers between tertiles of 'fruit and vegetable' and 'fats and meat' patterns.

**Table 5.5 Metabolic and endocrine biomarkers of dietary pattern tertiles**

	Refined and processed pattern			Sweet and savoury snacking pattern			Fruit and vegetable pattern			Fats and meat pattern		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
Leptin (ng/mL)	5.50 ±0.001	8.51 ±0.001 <sup>aa</sup>	10.96 ±0.001 <sup>bbb</sup>	7.24 ±0.001	9.33 ±0.001	10.00 ±0.001 <sup>b</sup>	9.12 ±0.001	8.91 ±0.001	7.41 ±0.001	7.94 ±0.001	8.51 ±0.001	8.91 ±0.001
Ghrelin (pg/mL)	59.83 ±5.93	42.56 ±4.44	40.96 ±3.69 <sup>b</sup>	46.14 ±3.61	49.15 ±4.24	41.20 ±4.82	44.21 ±3.73	45.79 ±4.36	47.26 ±4.25	44.24 ±4.54	49.49 ±4.09	42.39 ±3.74
Insulin (3–25 mU/mL) <sup>d</sup>	9.52 ±1.12	12.94 ±0.84 <sup>a</sup>	16.50 ±0.70 <sup>bbb cc</sup>	13.43 ±0.71	14.52 ±0.84	13.85 ±0.95	14.78 ±0.72	13.52 ±0.85	13.17 ±0.83	13.37 ±0.88	13.88 ±0.79	14.06 ±0.73
Glucose (3.5–5.4 mmol/L) <sup>d</sup>	4.66 ±0.06	4.76 ±0.05	4.76 ±0.04	4.74 ±0.04	4.77 ±0.04	4.64 ±0.05	4.76 ±0.04	4.72 ±0.04	4.66 ±0.04	4.66 ±0.05	4.73 ±0.04	4.80 ±0.04
HbA1c (<40 mmol/mol) <sup>d</sup>	28.81 ±0.52	29.05 ±0.39	29.89 ±0.32	29.44 ±0.32	29.27 ±0.37	29.46 ±0.42	29.62 ±0.32	28.91 ±0.38	29.51 ±0.37	29.48 ±0.39	29.64 ±0.35	29.22 ±0.32
Cholesterol (<5 mmol/L) <sup>d</sup>	4.59 ±0.13	4.51 ±0.10	4.45 ±0.08	4.51 ±0.08	4.45 ±0.10	4.39 ±0.11	4.40 ±0.08	4.52 ±0.10	4.44 ±0.10	4.31 ±0.10	4.54 ±0.09	4.54 ±0.08
HDL-C (>1 mmol/L) <sup>d</sup>	1.73 ±0.06	1.46 ±0.04 <sup>aaa</sup>	1.41 ±0.04 <sup>bbb</sup>	1.49 ±0.04	1.54 ±0.04	1.46 ±0.05	1.50 ±0.04	1.48 ±0.04	1.52 ±0.04	1.51 ±0.05	1.50 ±0.04	1.52 ±0.04
LDL-C (0–3.4 mmol/L) <sup>d</sup>	2.49 ±0.13	2.57 ±0.09	2.59 ±0.08	2.59 ±0.08	2.46 ±0.09	2.48 ±0.10	2.53 ±0.08	2.55 ±0.09	2.48 ±0.09	2.38 ±0.10	2.61 ±0.09	2.56 ±0.08
Triglyceride (<2 mmol/L) <sup>d</sup>	0.82 ±0.03	1.07 ±0.08 <sup>a</sup>	1.00 ±0.05	0.95 ±0.05	0.98 ±0.08	0.95 ±0.04	0.90 ±0.04	1.05 ±0.08	0.94 ±0.05	0.92 ±0.04	0.97 ±0.08	1.00 ±0.05

	Refined and processed pattern			Sweet and savoury snacking pattern			Fruit and vegetable pattern			Fats and meat pattern		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
CRP (0–5 mg/L) <sup>d</sup>	3.31 ±1.07	3.72 ±1.05	3.47 ±1.05	3.39 ±1.05	3.39 ±1.05	3.80 ±1.05	3.47 ±1.05	3.63 ±1.05	3.47 ±1.05	3.47 ±1.05	3.39 ±1.05	3.55 ±1.05
IL-6 (pg/mL)	1.95 ±1.10	2.09 ±1.07	2.14 ±1.05	2.00 ±1.05	1.95 ±1.07	2.34 ±1.07	2.34 ±1.05	2.00 ±1.07	1.86 ±1.07	2.14 ±1.07	1.95 ±1.07	2.04 ±1.07
IL-10 (pg/mL)	12.59 ±1.17	9.55 ±1.12	12.02 ±1.10	10.47 ±1.10	10.72 ±1.12	13.49 ±1.12	11.22 ±1.10	12.59 ±1.12	10.72 ±1.12	13.49 ±1.12	9.33 ±1.10	10.96 ±1.10
TNF-α (pg/mL)	6.81 ±0.37	7.35 ±0.28	7.36 ±0.23	7.18 ±0.23	7.36 ±0.27	7.07 ±0.31	7.41 ±0.23	7.42 ±0.27	6.61 ±0.26	7.14 ±0.29	7.11 ±0.26	7.12 ±0.24

T1, T2, T3: dietary pattern tertiles 1,2 and 3. HbA1c: glycosylated haemoglobin, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, CRP: C-reactive protein, IL-6: interleukin-6, IL-10: interleukin 10, TNF-α: tumour necrosis factor-alpha. *n*=126 in each tertile. Data reported as estimated marginal means ± SEM. Differences between tertiles tested by analysis of covariate (covariates: ethnic group, age) and post hoc test (with a Bonferroni correction).

<sup>a</sup> Significantly different between tertiles 1 and 2, <sup>a</sup>*p*<0.05, <sup>aa</sup>*p*<0.01, <sup>aaa</sup>*p*<0.001.

<sup>b</sup> Significantly different between tertiles 1 and 3, <sup>b</sup>*p*<0.05, <sup>bb</sup>*p*<0.01, <sup>bbb</sup>*p*<0.001.

<sup>c</sup> Significantly different between tertiles 2 and 3, <sup>c</sup>*p*<0.05, <sup>cc</sup>*p*<0.01, <sup>ccc</sup>*p*<0.001.

<sup>d</sup> Normal healthy ranges for metabolic biomarkers (New Zealand Guidelines Group., 2012).

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## 5.5 Discussion

The overall objective of the present study was to explore links between dietary patterns, body composition, macronutrient intakes and metabolic and endocrine biomarkers in women from three ethnic groups with different metabolic disease and obesity risk. There were two main findings in the present study. Firstly, in our group of healthy, pre-menopausal women, the differences in body composition and endocrine regulators of adiposity and appetite between the tertiles of the 'refined and processed' dietary pattern suggest a pathway to diet-induced metabolic dysregulation. Secondly, we observed a clear ethnic group-specific difference in dietary patterns where more Māori and Pacific women followed the 'refined and processed' pattern and more NZE women followed the 'sweet and savoury snacking' pattern. The ethnic group-based differences in dietary patterns highlight the need to understand broader determinants (e.g., socio-economic and lifestyle factors) that may influence the type of diets chosen by different ethnic groups.

### 5.5.1 Diet-induced metabolic dysregulation associated with the 'refined and processed' dietary pattern

The 'refined and processed' pattern of the present study was characterised by foods that are refined, highly processed and energy-dense (e.g., crumbed and deep-fried food, fast-food, puddings, fruit drinks, soft drinks). Consistent with findings of others, we found that women with higher scores for the 'refined and processed' pattern had higher intakes of total energy, percentage carbohydrate, starch, total sugar and sucrose and a lower intake of percentage protein compared to women with lower scores (Heidemann *et al.*, 2011; Naja *et al.*, 2013). We also found that women with higher scores for the 'refined and processed' pattern had a lower percentage of fat intake, in contrast to studies that report higher total fat and saturated fat intakes associated with similar dietary patterns (Heidemann *et al.*, 2011; Naja *et al.*, 2013). In our study, it is not surprising that women with higher intakes of the 'refined and processed' pattern had lower protein and fat intakes, as food groups characteristic to the dietary pattern are generally high in starch and sucrose. The higher fat intake could be explained by the additional food groups such as red meat, processed meat and butter present in dietary patterns of others (Heidemann *et al.*, 2011; Naja *et al.*, 2013). In our study, although women with higher scores for the 'refined and processed' pattern consumed a lower percentage of fat (34%) compared to women with lower scores (39%), fat intake of all tertiles were closer to or above the upper limit of the AMDR, indicating a diet generally high in fat (National Health and Medical Research Council, 2006).

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We found that women with higher scores for the 'refined and processed' pattern were younger and had higher body composition and anthropometric measurements (e.g., BMI, total and android fat, WC, HC) than women with lower scores, even after controlling for energy intake. Furthermore, women with higher scores for the 'refined and processed' pattern had higher circulating concentrations of insulin and leptin and lower plasma levels of ghrelin and HDL-C compared to women with lower scores. It has been proposed that refined carbohydrates in general and sugars in particular promote hyper-insulinaemia, which in turn drives glucose and fatty acids into adipose tissue, resulting in preferential deposit of ingested calories as fat (Thompson *et al.*, 2007). Our data suggest that higher energy and carbohydrate intakes (starch and sugar) in women with higher scores for the 'refined and processed' pattern may drive hyper-insulin secretion and increased adiposity, leading to hyper-leptinaemia and leptin resistance, as reported in previous studies describing the pathogenesis of obesity (Saltiel, 2012; Schwartz *et al.*, 2017). In the present study, it is important to note that the increase in insulin secretion appears to be sufficient to achieve normal plasma glucose and HbA1c concentrations (New Zealand Guidelines Group., 2012).

While higher plasma leptin and concomitantly lower plasma ghrelin levels have been observed in the obese state previously (Carlson *et al.*, 2009), this is the first study to show lower ghrelin concentrations in women with higher scores for the 'refined and processed' pattern. It is tempting to speculate that lower plasma ghrelin concentrations may represent a physiological adaptation to reduce appetite to the high intake of refined carbohydrates and sugar. However, the reduction in plasma ghrelin may not be sufficient to reduce appetite in the face of hyper-leptinaemia and developing leptin resistance (Krechowec *et al.*, 2006). These changes in endocrine regulators including insulin, leptin and ghrelin, disturb appetite regulation such that the energy balance is biased towards further fat accumulation and metabolic disease progression (Schwartz *et al.*, 2017). Furthermore, lower circulating concentrations of HDL-C in women with higher intakes of the 'refined and processed' pattern may represent an early indication of disturbed lipid metabolism (Barter, 2005). However, we did not observe differences in any other lipid or inflammatory markers between the tertiles of the 'refined and processed' pattern. The lack of biomarker differences may be attributed to the characteristics of the study participants, that is, they were generally healthy with no prior chronic illness.

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### 5.5.2 Ethnic group-specific differences in dietary patterns

In our study there were significantly more Māori (51%) and Pacific (68%) women and significantly less NZE (16%) women following the ‘refined and processed’ pattern. Furthermore, significantly more NZE (40%) women and significantly less Māori (15%) women followed the ‘sweet and savoury snacking’ pattern, while significantly less NZE women (27%) and significantly more Māori women (47%) followed the ‘fats and meat’ pattern. These differences in dietary patterns between the ethnic groups indicate an ethnic group-specific difference in intake that requires further investigation to explore factors that influence dietary patterns.

Similar to the findings of our study, two other studies conducted in pregnant NZ women found that higher intakes of the ‘junk’ dietary pattern characterised by highly refined and processed foods was associated with Māori and Pacific ethnic groups (Wall *et al.*, 2016; Thompson *et al.*, 2010). In our study, we also observed higher levels of adiposity and plasma insulin levels in Māori and Pacific women. Furthermore, even though plasma glucose and HbA1c levels of all ethnic groups were within the normal healthy range (New Zealand Guidelines Group., 2012), Pacific women had higher glucose and HbA1c levels than NZE women, indicating an early sign of impaired glucose metabolism. We could speculate that the endocrine and metabolic consequences of consuming dietary patterns such as the ‘refined and processed’ pattern may be a significant contributor of higher metabolic disease and obesity risk profiles observed in Māori and Pacific ethnic groups (Ministry of Health, 2017).

More importantly, ethnic group-specific differences in dietary patterns highlight the need to explore the impact of broader determinants of health that influence the types of food chosen by different ethnic groups. The 2008/09 Adult Nutrition Survey found that Māori and Pacific families were more likely to experience food insecurity (when the availability of nutritionally adequate and safe foods, or the ability to acquire such foods is limited) than other NZ population groups (Ministry of Health, 2011). Furthermore, it is known that the cost of food is a major determinant of food choices (Glanz *et al.*, 1998; Drewnowski and Darmon, 2005). Socio-economic inequities (e.g., income, employment, housing, education) have been clearly documented for Māori and Pacific ethnic groups in comparison to other NZ population groups (Southwick *et al.*, 2012; Ministry of Health, 2015c; Reremoana *et al.*, 2015). Therefore, the differences in dietary patterns observed between the ethnic groups in the present study are most likely related to the differences in socio-economic determinants which amplifies the health inequities between ethnic groups (Southwick *et al.*, 2012; Ministry of Health, 2015c). In particular, the finding that more Māori and Pacific women were following the ‘refined and

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processed' dietary pattern which characterises foods that are easily accessible and more affordable but energy-dense and often nutrient-poor may explain, at least in part, the differences in obesity and metabolic disease risk influenced by socio-economic aspects (Luiten *et al.*, 2015; Drewnowski and Darmon, 2005; Rao *et al.*, 2013). This highlights the need to investigate the link between dietary patterns and socio-economic aspects that may be linked to inequality in access to healthy food options, to understand specific contributors and drivers of obesity within each ethnic group in order to reduce the progression of obesity and improve metabolic health.

### **5.5.3 Relationship between other dietary patterns and metabolic health**

In our study, we did not observe any differences in age, ethnic group or body composition between the tertiles of the 'fruit and vegetable' pattern. This is in contrast to the findings of one study that showed an inverse association between the 'fruit, vegetables and dairy' pattern and BMI, WC and blood pressure (McNaughton *et al.*, 2007) and another study that showed higher intake of the 'healthy' pattern to be associated with lower WCs (Suliga *et al.*, 2015). The differences in findings between our study and others could be attributed to the additional food groups (e.g., dairy, fish, poultry) present in the 'healthy' and 'Prudent' dietary patterns, which constitutes food groups of a more balanced diet (Fung *et al.*, 2001; Newby *et al.*, 2006). We could also speculate that in our study, women with higher intakes of the 'fruit and vegetable' pattern may also have higher physical activity levels and thereby maintain normal weight. Furthermore, although total sugar was higher in women with higher intakes, total sugar mostly consisted of natural sugars from fruits and vegetables (i.e., fructose) rather than added sugars (i.e., sucrose). In addition, we did not observe any differences in metabolic markers of appetite, glucose homeostasis, lipid profile and inflammation, which further supports the lack of differences in body composition measurements between tertiles of the 'fruit and vegetable' pattern in this study.

In the present study, women with higher scores for the 'sweet and savoury snacking' and 'fats and meat' dietary patterns had higher body weight, total body fat %, WC and HC than women with lower scores. However, after adjusting for energy intake there were no significant differences in body composition measurements between the tertiles of the both dietary patterns. Furthermore, we did not observe any differences in metabolic biomarkers between the dietary pattern tertiles. The lack of body composition and metabolic biomarker differences between the tertiles of these dietary patterns could be attributed to the lower variance in food intake explained by these patterns, as it has been discussed that dietary patterns that explain

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larger variances in food intake more strongly relate to health outcomes (Randall et al., 1990; Schulze et al., 2007).

#### **5.5.4 Strengths, limitations and future studies**

The present study has several strengths. Dietary patterns in our study were derived using a well-established data-driven statistical method. The dietary patterns identified and the variance in food intake explained by the different patterns were similar to other studies (Paradis *et al.*, 2009; Newby *et al.*, 2004; Suliga *et al.*, 2015). For example, in our study, the 'refined and processed' dietary pattern explained 9% of the variance in food intake and was similar to the 'Western' pattern which explained 10% of the variance (Paradis *et al.*, 2009) and the fast-food/dessert' (Naja *et al.*, 2013) and 'refined grains and dessert' patterns (Naja *et al.*, 2012) which explained 13% of the variance in food intake. Furthermore, the total percentage variance explained by the four dietary patterns of our study (25%) was similar to a study done in pregnant NZ women that identified four dietary patterns (junk, health conscious, traditional/white bread, fusion/protein) explaining 23.4% variance (Wall *et al.*, 2016), but higher than a recent study in NZ men and women that identified two dietary patterns (healthy, traditional) explaining 12% of the variance in food intake (Beck *et al.*, 2017). The percent variance explained by individual dietary patterns is a function of the number of variables included in the factor analysis (i.e., food groups) and the correlation matrix itself (Schwerin *et al.*, 1981; Kim and Mueller, 1978). Therefore, we would expect differences in variances explained by the different dietary patterns between studies. We also used several measures of body composition (BMI, total and regional body fat) and a range of metabolic and endocrine biomarkers of adiposity, appetite and inflammation to assess the link between dietary patterns and metabolic health. Previous NZ studies have only investigated associations between dietary patterns, socio-demographic factors, lifestyle factors and a limited number of anthropometric measurements (e.g., BMI, waist circumference) (Beck *et al.*, 2017; Wall *et al.*, 2016; Thompson *et al.*, 2010; Schrijvers *et al.*, 2016).

There are several limitations that should be addressed in future studies. Firstly, the study population was a convenience sample of adult women living in Auckland and therefore the findings between dietary patterns, body composition and metabolic biomarkers cannot be generalised to other groups. Therefore, future studies should explore these relationships in other populations such as in men and in different age, BMI and ethnic groups. Secondly, the study participants were generally healthy (due to the exclusion criteria) and free of any chronic illness. We believe these participant characteristics may have contributed to the lack of

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associations between some dietary patterns and metabolic biomarkers. It has been discussed that measuring the concentrations of inflammatory biomarkers under basal conditions is probably less informative compared with the biomarker concentration changes in response to a challenge (e.g., nutritional intervention) (Calder *et al.*, 2013). Therefore, future studies should investigate the inflammatory responses to dietary and other physiological challenges that would provide a better indication of the impact of nutrition on inflammatory homeostasis between different population groups.

Due to the significant challenges encountered during recruitment, the sample sizes of Māori and Pacific women were smaller than that of the NZE women. Nevertheless, we observed clear ethnic group-based differences in dietary patterns. Future studies should investigate the link between diet and health outcomes taking into account the broader determinants of health within each ethnic group separately. Furthermore, other dietary factors (e.g., eating behaviour factors) and non-dietary factors (physical activity, smoking, alcohol intake, education, income) influence food intake, and future studies should control for these variables when assessing links between dietary patterns and metabolic health. Lastly, due to the cross-sectional nature of the study only indications of relationships could be ascertained, and follow-up studies are needed to further evaluate whether certain dietary patterns lead to changes in body composition and metabolic health outcomes.

## 5.6 Conclusions

The differences in body composition and endocrine regulators across the tertiles of the 'refined and processed' dietary pattern can explain, at least in part, the aetiology of obesity and characterises a trajectory that may lead to metabolic disease. The 'refined and processed' pattern is also representative of foods that are refined, highly processed, energy-dense and rich in carbohydrate (starch and sucrose), reflecting an unhealthy dietary composition. The findings of our study call for evidence-based recommendations to limit the consumption of refined and processed foods such as crumbed and deep-fried food, fast-food, sweetened beverages, sweetened milk products and refined grains. Although such guidelines are already implemented (e.g., Eating and Activity Guidelines for New Zealand adults) (Ministry of Health, 2015b), the findings of this study emphasise the need to promote this healthy eating message further through public health initiatives (e.g., education at preschools and schools, removing sugary drinks from vending machines) (Ministry of Education, 2014; Gorton *et al.*, 2010; Thornley and Sundborn, 2014).

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Another important finding of this study was the ethnic group-specific differences in dietary patterns. The finding that more Māori and Pacific women were following the ‘refined and processed’ dietary pattern highlights the need for further investigation into the link between dietary patterns and socio-economic parameters that may amplify the health inequities between populations groups (Ministry of Health, 2015c; Southwick *et al.*, 2012). By doing so, specific contributors and drivers of obesity within each ethnic group can be better understood. Furthermore, the findings of this study support the need to create a healthier food environment and to make healthy foods more affordable for all population groups.

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## Chapter 6

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### ***Are Patterns of Sweet and Fat (Creaminess) Hedonic Liking Associated with Dietary Intake?***

#### **6.1 Abstract**

Although past studies have identified distinct patterns of sweet hedonic liking, the relationship between these patterns and dietary intake is not well explored. The overall aim of our study was to investigate whether body composition, dietary intake and metabolic and endocrine biomarkers differ between women with distinct patterns of sweet and fat (creaminess) hedonic liking. A total of 408 women between the ages of 16–45 years were recruited. Participants rated on a gLMS, the sweet taste and fat (creaminess) intensity and hedonic liking of five sucrose and milk samples respectively. Patterns of sweet and creaminess hedonic liking were determined using the hierarchical cluster analysis method. A 220-item FFQ was used to determine dietary intake. Several parameters of body composition and metabolic and endocrine biomarkers (e.g., glucose, leptin) were also measured. Cluster analysis revealed two distinct patterns of sweet and creaminess hedonic liking. Twenty-seven percent of women were sweet likers and 73% were sweet dis-likers. Forty-five percent of women were creaminess likers and 55% were creaminess dis-likers. Apart from sweet likers being younger than sweet dis-likers ( $p=0.01$ ), no other differences in age and body composition were found between the hedonic liking patterns. Interestingly, sweet likers had higher frequency of intakes of sweet tasting food groups (e.g., sweet snack foods, cakes and biscuits) ( $p<0.01$ ) and higher intakes of the ‘refined and processed’ dietary pattern compared to sweet dis-likers ( $p=0.007$ ). Creaminess likers had higher frequency of intakes of fatty tasting food groups (e.g., fats, processed meats) ( $p<0.01$ ) and a higher intake of the ‘fats and meat’ dietary pattern compared to creaminess dis-likers ( $p=0.02$ ). Our study shows that there are distinct patterns of sweet and fat hedonic liking. Furthermore, higher hedonic liking for sweet and fat tastes are linked with increased intakes of sweet and fatty tasting food groups and dietary patterns such as the ‘refined and processed’ and ‘fats and meat’ patterns.

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## 6.2 Introduction

The increased availability and over-consumption of energy-dense, nutrient-poor processed foods that are high in sugar and fat have paralleled the increase in obesity and metabolic disease rates (Popkin and Gordon-Larsen, 2004; Monteiro *et al.*, 2013; Vandevijvere and Swinburn, 2014). There is ample evidence linking excess sugar and fat intakes with a myriad of adverse health consequences, including weight gain, obesity, cardiovascular disease and type 2 diabetes (Te Morenga *et al.*, 2013; Malik *et al.*, 2006; Bray and Popkin, 1998; Han *et al.*, 2015). Furthermore, following dietary patterns such as the ‘Western’ or ‘unhealthy’ patterns characterised by high intakes of refined grains, fast-food, processed food and soft drinks have been associated with higher body weight, higher BMI and increased adiposity (Paradis *et al.*, 2009). Foods rich in sugar and fat are extremely pleasurable, energy-dense and highly palatable. The sensory properties associated with sweet and fat tastes, in particular the positive hedonic liking can promote over-consumption leading to excess energy intake (Deglaire *et al.*, 2015; Drewnowski, 1997b).

The sense of taste is an important driver of food choice as it directs us towards particular food and influences the amount of food consumed (McCrickerd and Forde, 2016). However, there are large inter-individual differences in the ability to detect and perceive sweet and fat tastes and in the extent to which these tastes are liked (Low *et al.*, 2016; Kim *et al.*, 2014; Mennella *et al.*, 2012). Many questionnaire-based studies have shown that higher hedonic liking for sweet and fat tastes might be a risk factor for the development of obesity, largely explained by higher intakes of sugar- and fat-rich foods (Lampur e *et al.*, 2016; Deglaire *et al.*, 2015). Therefore, it is important to quantify inter-individual differences in sweet and fat hedonic liking to understand how these differences influence dietary intake, metabolic regulation and long-term health.

Hedonic liking is commonly assessed using laboratory-based chemosensory tests such as rating the hedonic liking of different tastant concentrations or using a paired comparison method to select the most preferred tastant concentration (Mennella *et al.*, 2011; Drewnowski *et al.*, 1999). Although valid and reliable, these methods assume that an individual’s hedonic liking is highest at one concentration. Past studies have identified different patterns of sweet taste hedonic responses. Some of the early work undertaken in this area identified two types of response curves for sweet pleasantness ratings (Moskowitz *et al.*, 1974; Thompson *et al.*, 1976; Johnson *et al.*, 1979). Type I hedonic response curves were characterised by an inverted U shape pattern, where sweet pleasantness ratings increase with increasing sucrose concentrations (0.06–0.6 M) and decrease after a peak of pleasantness is reached (>0.6 M). Type II response

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curves show a monotonic increase in pleasantness with increasing sucrose concentrations and reach a plateau beyond the highest concentration (Thompson *et al.*, 1976).

Most past studies have classified individuals as sweet likers or sweet dis-likers by manually assigning individuals into the relevant groups (Yeomans *et al.*, 2007; Looy and Weingarten, 1991). More recently, cluster analysis has been used to classify individuals according to their patterns of sweet hedonic liking (Asao *et al.*, 2015; Kim *et al.*, 2014). Cluster analysis is an explorative data-driven statistical method used to aggregate individuals with similar hedonic liking patterns into non-overlapping groups (i.e., clusters). Each individual belongs to one cluster and further data analysis can be performed to assess differences in measured variables between clusters (e.g., body composition, dietary measurements) (Field, 2009). Using the cluster analysis method, Asao *et al.* (2015) identified a group of individuals who liked the sweetness of low sucrose concentrations and another group that liked the sweetness of high sucrose concentrations (Asao *et al.*, 2015). Kim *et al.* (2014) identified similar sweet hedonic likings patterns and also found that sweet likers had higher liking ratings for sweet foods (e.g., chocolate flavoured milk, donuts), suggesting a positive association between sweet hedonic liking and sweet food liking (Kim *et al.*, 2014). With regards to fat taste, a recent study identified three distinct patterns of fat hedonic liking and found that high-fat likers had significantly higher dairy intakes compared to high-fat dis-likers (Shen *et al.*, 2017). Apart from a few studies reporting a positive relationship between sweet and fat hedonic liking and liking for sweet and fatty foods, there is a dearth of information whether there is a link between patterns of sweet and fat hedonic liking and dietary intake.

The present study had two main aims. The first aim was to identify and characterise patterns of sweet and fat (creaminess) hedonic liking using the hierarchical cluster analysis method. The second aim was to explore whether body composition, frequency of food group intake, dietary patterns and metabolic and endocrine biomarkers of adiposity and appetite differ between women with distinct patterns of sweet and fat (creaminess) hedonic liking. The findings of the present study further our understanding of the link between sweet and fat hedonic liking, dietary intake, body composition and metabolic health profiles.

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## 6.3 Methods

### 6.3.1 Study design and participants

The data used in the present study are part of the women's EXPLORE study, a cross-sectional study designed to investigate predictors and metabolic disease risks associated with different body fat profiles of New Zealand women (Kruger *et al.*, 2015). All procedures of the EXPLORE study were reviewed and approved by the Massey University Human Ethics Committee (Southern A, Application 13/13). The EXPLORE study was registered in the Australian New Zealand Clinical Trials Registry as ACTRN12613000714785. In total, 408 post-menarche and pre-menopausal NZE ( $n=233$ ), Māori ( $n=84$ ) and Pacific ( $n=91$ ) women between the ages of 16–45 years were recruited. Māori and Pacific ethnic groups were defined by self-identification and having at least one parent from the same ethnic group (Kruger *et al.*, 2015). The exclusion criteria were; currently pregnant or breastfeeding, irregular menstrual cycles, diagnosed with any chronic illness, allergic to dairy products or taking medication that can alter taste perception (Steinbach *et al.*, 2009; Redda and Allis, 2006). Participants were recruited from the Auckland area through advertisements, posters and flyers at various venues, social media, email lists and community groups. Culturally appropriate recruitment methods were used for Māori and Pacific ethnic groups. Written informed consent was obtained from all women prior to participating in the study.

### 6.3.2 Study procedure

The EXPLORE study was conducted in two phases. Phase one of the study involved screening participants for eligibility and phase two involved all data collection. Participants who expressed interest completed a screening questionnaire to assess their initial eligibility of age, ethnic group and health. Women who fit the inclusion criteria were then invited to participate in the screening phase at the Human Nutrition Research Unit at Massey University in Auckland, or at an off-site location convenient to the participants. Participant's height using a portable stadiometer, and weight and body fat using the bioelectrical impedance analysis machine (Biospace, Inbody 230, Cerritos, California, USA) were obtained. Participant's eligibility was assessed using their BMI and body fat % as required by the EXPLORE study design (Kruger *et al.*, 2015). Women who met eligibility were invited to participate in phase two of the study.

All phase two data collection was conducted at the Human Nutrition Research Unit and Sensory Research Facility at Massey University in Auckland. To standardise hunger and diurnal hormonal fluctuations (Gavrila *et al.*, 2003), all testing appointments were organised following an

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overnight fast between 7–9.30 a.m. Participants were asked to refrain from brushing their teeth and performing any physical activity an hour prior to testing. Furthermore, phase two appointments were scheduled during the first 14 days of the menstrual cycle to standardise effects of menstrual cycle hormones on taste perception and energy intake (Davidsen *et al.*, 2007) and to ensure participants were not pregnant at the time of data collection. In the present study, only methods associated with sweet taste and fat (creaminess) perception, body composition, metabolic biomarkers and dietary intake will be discussed.

### **6.3.3 Sweet taste and fat (creaminess) perception measurements**

Sweet taste perception was assessed by rating the sweet taste intensity and sweet hedonic liking of 20 mM (0.6% w/v), 90 mM (3% w/v), 180 mM (6% w/v), 360 mM (12% w/v) and 720 mM (24% w/v) sucrose samples prepared in distilled water (Holt *et al.*, 2000). Fat taste perception was assessed by rating the creaminess intensity and creaminess hedonic liking of five milk samples with varying levels of fat percentages; 0.1, 1.5, 3.3, 18.5 and 36.9% (Salbe *et al.*, 2004). All sucrose samples were prepared on the day of testing and presented at room temperature (Asao *et al.*, 2015), while milk samples, also prepared on the day of testing, were refrigerated and presented at 4° C (Proserpio *et al.*, 2017). Sensory testing was conducted under red light in individual sensory booths set to room temperature (20° C). Participants were asked to take the whole sample (10 ml) into their mouth, swirl it around for a few seconds and swallow the sample (Mahar and Duizer, 2007). Studies conducted in our laboratory indicated that participants were uncomfortable with expectorating the tastant samples. Therefore, to maintain compliance with the sensory methodology all participants were asked to swallow the tastant samples similar to previous publications (Mahar and Duizer, 2007; Holt *et al.*, 2000). All five samples were presented in a random order starting with sucrose followed by the milk samples. Participants were instructed to rinse their mouth with distilled water between samples. Nose clips were not worn during any taste measurement to emulate the sensory perceptions experienced during normal eating circumstances.

Perceived intensity was rated on a 100 mm gLMS ranging from no sensation (0 mm) to strongest imaginable sensation (100 mm) with verbal descriptors assigned to different levels of intensities (Green *et al.*, 1996). Hedonic liking was rated on a hedonic gLMS ranging from dislike extremely (-100 mm) to like extremely (+100 mm) with neutral (0 mm) in the middle (Lim *et al.*, 2009). Participants were instructed on how to use both scales according to the protocol outlined by Green *et al.* (1996) and were encouraged to mark anywhere on the scale as seemed appropriate.

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For example, if a sample was perceived between moderate and strong, they were asked to mark closer to the verbal descriptor that more closely represented the taste sensation perceived.

### **6.3.4 Body composition measurements**

Participant's BMI was determined by measuring their weight to the nearest 0.1 kg using an electronic scale and height to the nearest 0.1 cm using a Harpenden stadiometer. Total body fat was measured by full body composition analysis using the air displacement plethysmography device and thoracic gas volume method (BodPod, 2007A, V4.2+ software, Life Measurement Inc., Concord, California, USA) (Wingfield *et al.*, 2014).

### **6.3.5 Metabolic and endocrine biomarkers**

Fasting blood samples were collected in EDTA and serum vacutainer tubes by qualified phlebotomists. An aliquot of EDTA whole blood was immediately frozen at -80° C for HbA1c analysis. Within an hour of collection, vacutainer tubes were centrifuged at 3500 rpm at 4° C for 15 minutes and aliquots were frozen at -80° C. Blood samples were used to measure metabolic biomarkers of appetite regulation (leptin, ghrelin), glucose homeostasis (insulin, glucose, HbA1c), lipid profile (cholesterol, HDL-C, LDL-C, triglyceride) and inflammation (IL-6, IL-10, TNF- $\alpha$ , CRP).

Serum insulin was measured by the automated analyser ADVIA Centaur system using a chemiluminescent two-site sandwich immunoassay method (Siemens Healthcare Diagnostics, catalogue # 02230141-128434) (Schiaffini *et al.*, 2010). Using standard automated laboratory procedures of the Dimension Vista system (Siemens Healthcare Diagnostics), serum levels of cholesterol (cholesterol esterase and cholesterol oxidase method, catalogue # K1027) (Dirinck *et al.*, 2015), CRP (catalogue # K7032), HDL-C (cholesterol oxidase and cholesterol esterase method, catalogue # K3048) (Tosi *et al.*, 2016), glucose (hexokinase and glucose-6-phosphate dehydrogenase method, catalogue # K1039) (Olson *et al.*, 2012) and triglyceride (lipase and glycerol kinase method, catalogue # K2069) (Dirinck *et al.*, 2015) were measured. Serum LDL-C was calculated using measured variables. The HbA1c levels of EDTA whole blood were measured using the high performance liquid chromatography method (Biorad Variant instrumentation, USA) (Kopprasch *et al.*, 2009). Milliplex immunoassay kits (Millipore Corp., Billerica, Massachusetts, USA) were used to simultaneously measure plasma levels of IL-6, IL-10 and TNF- $\alpha$ , (catalogue # HSTCMAG-28SK), and leptin and ghrelin (catalogue # HMHEMAG-34K) as previously described (Gabel *et al.*, 2016).

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### **6.3.6 Dietary measurements**

All participants completed a validated 220-item semi-quantitative FFQ to measure habitual intakes of foods and beverages consumed over the previous month (Appendix 5) (Beck *et al.*, 2018). The FFQ contained food items organised by common food groups (e.g., breads and cereals, fish and seafood, fruits and vegetables) and included predominantly sweet tasting (e.g., sweet snacks, soft drinks, sweetened milk products, sweet spreads) and creamy/fatty tasting (e.g., processed meat, fats, dressings and sauces) food groups. The intakes of the food items were determined using standard natural portions (e.g., 1 medium apple, 1 muffin, 2 biscuits) or standard weights and volumes (e.g., 1 pottle of yoghurt, 1 sausage, 1 beef patty). Participants were required to indicate on average how many times in the previous month they consumed each of the food item in relation to the standard serving size. The frequency of intake categories were; never, less than once per month, 1–3 times per month, once per week, 2–3 times per week, 4–6 times per week, once a day, 2–3 times per day or more than four times per day. The food items in the FFQ were adapted from the 2008/09 New Zealand Adult Nutrition Survey (Ministry of Health, 1997; Ministry of Health, 2011).

### **6.3.7 Data handling and statistical analysis**

#### **6.3.7.1 Incomplete data**

A total of 408 participants completed the study. Following blood sample analysis, we excluded two participants as their HbA1c levels were higher than 50 mmol/mol (New Zealand Guidelines Group., 2012). A further eight participants had no taste perception data and were excluded from the analysis. Of the remaining 398 participants, complete sweet and creaminess perception data were available for 393 and 387 participants respectively.

#### **6.3.7.2 Determining patterns of sweet and fat hedonic liking**

As discussed in Chapter 4, we observed no clear differences in sweet taste and fat (creaminess) perception between the ethnic groups in our study. Therefore, the pooled sample of all three ethnic groups was used to determine patterns of sweet and fat hedonic liking. Furthermore, sweet and creaminess perception was assessed separately instead of using a mixture of sweet and fat tastants together. Therefore, we believed that sweet and creaminess hedonic liking clusters should be determined for each taste separately, using each participant's hedonic liking ratings for the different tastes. Hierarchical cluster analysis (Ward's minimum variance method and Euclidean distances), an unsupervised classification method was used to identify clusters with similar patterns of sweet and creaminess hedonic liking (Asao *et al.*, 2015; Kim *et al.*, 2014).

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The hedonic liking patterns of the different clusters were visually confirmed. Two clusters each best described the patterns of sweet and creaminess hedonic liking for the study population.

### **6.3.7.3 Food frequency questionnaire data analysis and organisation of food groups**

All data obtained from the FFQ were entered in FoodWorks 7 (FoodWorks professional 2013, Xyris Software, Queensland, Australia) using the New Zealand Food Composite Database to determine energy and macronutrient intakes. Participants whose daily energy intakes were <1000 kcal or >5000 kcal (i.e., 4200–21,000 kJ) were excluded from the analysis (University of Otago and Ministry of Health, 2011; Willett, 2013). Four participants were excluded due to incomplete FFQ data and a further 10 and 14 participants were excluded due to under- and over-reporting respectively.

The original intake frequencies were converted to DFEs calculated by allocating proportional values to the original frequency categories with reference to a base value of 1.0, equivalent to once a day. The scores were calculated as follows: DFE score of 0—never; 0.008—less than once per month; 0.067—1–3 times per month; 0.14—once per week; 0.36—2–3 times per week; 0.71—4–6 times per week; 1—once a day; 2.5—2–3 times per day; 4—more than four times per day. The food items in the FFQ were then categorised into 57 food groups (Appendix 6) based on their nutritional composition and characteristics (e.g., full-fat milk, sweetened milk products, sweet snacks, red meats, processed meats). Some food items solely created a food group (e.g., potatoes, yoghurt) and were consumed by at least 10% of the study participants. For each participant, a single DFE score for each of the 57 food groups was calculated.

### **6.3.7.4 Dietary pattern extraction**

The in-depth details of dietary patterns are reported in Chapter 5. Briefly, dietary patterns were extracted using PCA and orthogonal rotation (Field, 2009). Using scree plots, an eigenvalue cut-off >1 and the factor loadings, four dietary patterns were derived. The factor loadings and food groups contributing to the different dietary patterns are found in Chapter 5, Table 5.2. The extracted dietary patterns were named based on the nutritional characteristics of the food groups.

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### 6.3.7.5 Statistical analysis

All data were analysed using SPSS software version 24 (IBM corporation, New York, USA). All continuous variables were tested for normality using Kolmogorov-Smirnov test together with analysing histograms and normal Q-Q plots. Non-normal data were log transformed and tested again for normality. Parametric data are reported as mean  $\pm$  SEM and non-parametric data as median [25,75 percentiles]. Categorical data are reported as *n* (%). The differences between two groups were tested using independent samples *t* test and Mann-Whitney *U* test for parametric and non-parametric data respectively (2-tailed). Repeated measures analysis of variance was used to test differences in sweet taste intensity ratings between sucrose samples and creaminess intensity ratings between milk samples. A  $p < 0.05$  was considered statistically significant.

## 6.4 Results

### 6.4.1 Participant characteristics

Characteristics of all participants and sweet and creaminess hedonic liking clusters are reported in Table 6.1. Due to significant challenges encountered during recruitment, the sample sizes of Māori and Pacific women were smaller than NZE women. Compared to the New Zealand statistics, the NZE group of the study (58%) was lower than the 74%, Māori (20%) higher than the 15% and Pacific (22%) higher than the 7% of the overall New Zealand population (Statistics New Zealand, 2013). As sweet taste and fat (creaminess) perception was assessed separately instead of using a mixture of sweet and fat tastants, we believed that hedonic liking clusters should be determined for each taste separately. Twenty-seven percent of women were identified as sweet likers and 73% were sweet dis-likers. Forty-five percent of women were identified as creaminess likers and 55% were creaminess dis-likers (Table 6.1). Sweet likers were slightly younger than sweet dis-likers ( $p = 0.01$ ), however body weight, BMI and body fat were not significantly different between patterns of sweet or creaminess hedonic liking ( $p > 0.05$ , Table 6.1). There were similar numbers of women from each ethnic group in each of the sweet and creaminess hedonic liking cluster. Furthermore, approximately 25% of women from each BMI and body fat group were sweet likers, while approximately half the women from each BMI and body fat group were creaminess likers (Table 6.1).

**Table 6.1 Characteristics of hedonic liking clusters**

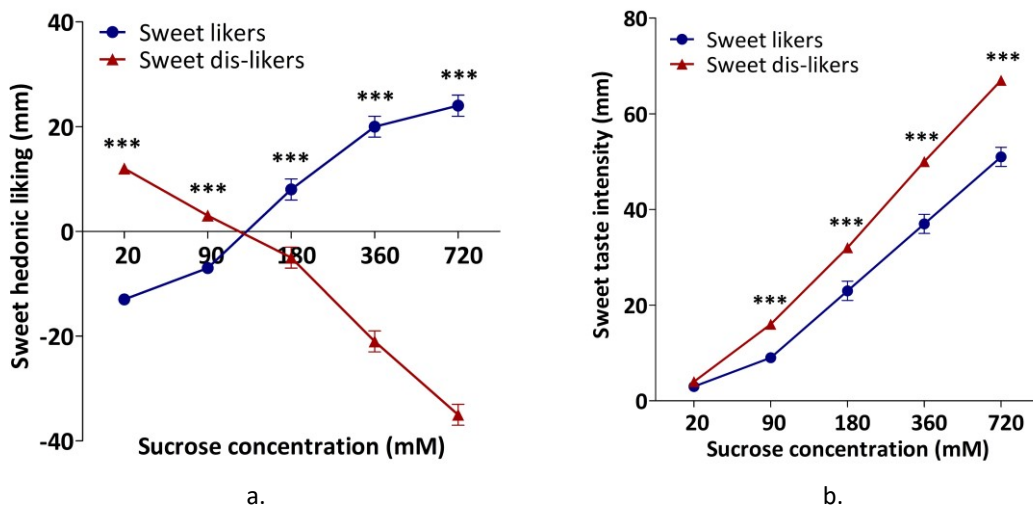
	All participants	Sweet hedonic liking clusters <sup>a</sup>		Creaminess hedonic liking clusters <sup>a</sup>	
		Sweet likers	Sweet dis-likers	Creaminess likers	Creaminess dis-likers
<i>n</i>	398	107	286	176	211
Age (years)	30.9±0.4	29.1±0.8	31.6±0.5 <sup>b</sup>	30.2±0.6	31.3±0.6
Ethnic group ( <i>n</i> (%))					
NZE	232(58%)	60(26%)	171 (74%)	98 (44%)	127 (56%)
Māori	80(20%)	21(27%)	57 (73%)	33 (42%)	45 (58%)
Pacific	86(22%)	26(31%)	58 (69%)	45 (54%)	39 (46%)
Body weight (kg)	75.9±0.9	77.0±1.8	75.4±1.0	76.9±1.4	75.4±1.2
BMI (kg/m <sup>2</sup> )	27.2±0.3	27.8±0.6	27.0±0.4	27.7±0.5	27.0±0.4
BMI groups <sup>c</sup> ( <i>n</i> (%))					
Normal-weight	179(45%)	43(24%)	133(76%)	76(44%)	97(56%)
Overweight	115(29%)	29(25%)	86(75%)	53(47%)	60(53%)
Obese	104(26%)	35(34%)	67(66%)	47(47%)	54(53%)
Total body fat (%)	34.2±0.4	34.7±0.8	33.9±0.5	34.2±0.6	34.1±0.6
Body fat groups ( <i>n</i> (%))					
<35%	225(57%)	55(25%)	167(75%)	97(44%)	121(56%)
>35%	173(43%)	52(30%)	119(70%)	79(47%)	90(53%)

NZE: New Zealand European, BMI: body mass index. Data reported as mean ± SEM or *n* (%). <sup>a</sup> Sweet and creaminess hedonic liking clusters were determined using the hierarchical cluster analysis method. <sup>b</sup> Significantly different between sweet likers and sweet dis-likers as determined by independent t test,  $p < 0.05$ . <sup>c</sup> BMI groups; normal-weight: 18.5–24.9 kg/m<sup>2</sup>, overweight: 25–29.9 kg/m<sup>2</sup>, obese: ≥30 kg/m<sup>2</sup> (World Health Organisation, 2000).

## 6.4.2 Patterns of hedonic liking

### 6.4.2.1 Patterns of sweet hedonic liking

The hierarchical cluster analysis method revealed two distinct clusters with significantly different sweet hedonic liking patterns. Sweet likers ( $n=107$ , 27%) were characterised by increasingly higher sweet hedonic liking ratings with increasing sucrose concentrations. Sweet dis-likers ( $n=286$ , 73%) were characterised by decreasing sweet hedonic liking ratings with increasing sucrose concentrations (Figure 6.1a). Sweet likers had significantly lower hedonic liking ratings for 20 mM and 90 mM sucrose and higher hedonic liking ratings for 180 mM, 360 mM and 720 mM sucrose compared to sweet dis-likers (all  $p<0.001$ , Figure 6.1a). As seen in Figure 6.1b, both sweet likers and dis-likers could differentiate between progressively increasing sucrose concentrations. However, sweet likers rated the sweetness of all sucrose concentrations (except 20 mM) as less intense compared to sweet dis-likers (all  $p<0.001$ ).

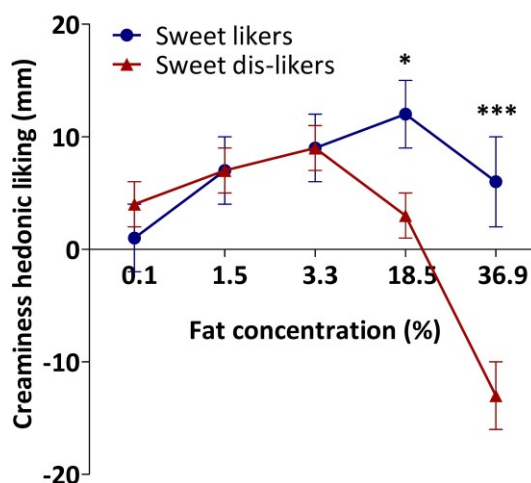


**Figure 6.1 Sweet taste perception ratings of sweet likers and dis-likers**

The graphs represent the mean sweet hedonic liking (a) and sweet taste intensity (b) ratings of sweet likers and sweet dis-likers. Data shown as mean  $\pm$  SEM. Sweet likers  $n=107$  and sweet dis-likers  $n=286$ . Hedonic liking clusters were determined using the hierarchical cluster analysis method. Differences between sweet hedonic liking clusters tested by independent t test, \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$ .

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Figure 6.2 shows the creaminess hedonic liking of sweet likers and sweet dis-likers. Interestingly, sweet likers had positive creaminess hedonic liking ratings across all milk samples while sweet dis-likers, disliked the creaminess of 36.9% milk sample. Independent t test revealed that sweet likers had significantly higher creaminess hedonic liking ratings for 18.5% ( $p=0.02$ ) and 36.9% ( $p<0.001$ ) milk samples compared to sweet dis-likers (Figure 6.2).

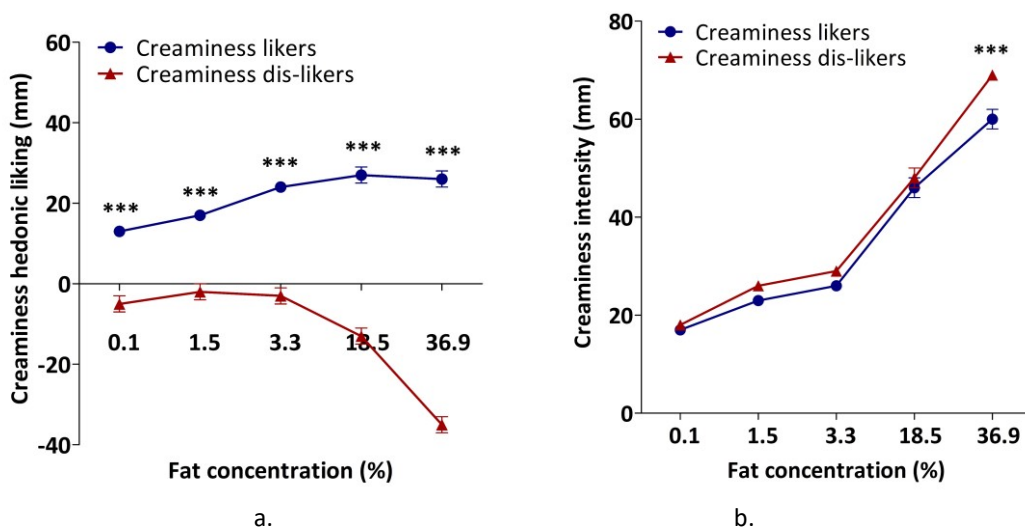


**Figure 6.2 Creaminess hedonic liking ratings of sweet likers and sweet dis-likers**

Data shown as mean  $\pm$  SEM. Sweet likers  $n=107$  and sweet dis-likers  $n=286$ . Differences between sweet hedonic liking clusters tested by independent t test, \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$ .

#### 6.4.2.2 Patterns of creaminess hedonic liking

Using the hierarchical cluster analysis method two distinct clusters with significantly different creaminess hedonic liking patterns were identified. Creaminess likers ( $n=176$ , 45%) were identified as the cluster with positive creaminess hedonic liking ratings across all milk samples and creaminess dis-likers ( $n=211$ , 55%) were identified by negative hedonic liking ratings across all milk samples (Figure 6.3a). Creaminess likers had significantly higher creaminess hedonic liking ratings across all milk samples compared to creaminess dis-likers (all  $p<0.001$ , Figure 6.3a). As seen in Figure 6.3b, both creaminess hedonic liking clusters perceived the increase in creaminess with increasing fat concentrations apart from 1.5% and 3.3% samples which were perceived the same. Interestingly, the only difference in creaminess intensity ratings between the creaminess hedonic liking clusters were observed at 36.9%, where creaminess likers perceived the 36.9% milk sample as less creamy than creaminess dis-likers ( $p<0.001$ , Figure 6.3b).

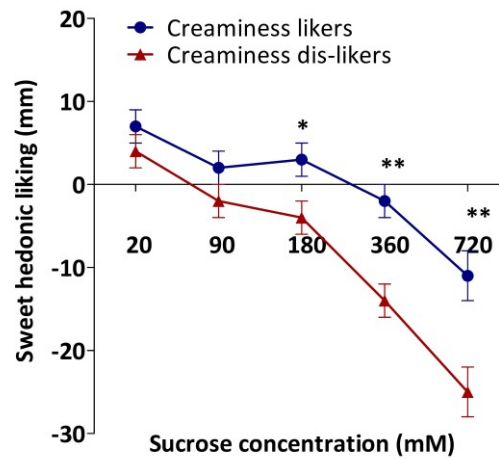


**Figure 6.3 Creaminess perception ratings of creaminess likers and dis-likers**

The graphs represent the mean creaminess hedonic liking (a) and creaminess intensity (b) ratings of creaminess likers and creaminess dis-likers. Data shown as mean  $\pm$  SEM. Creaminess likers  $n=176$  and creaminess dis-likers  $n=211$ . Clusters were determined using the hierarchical cluster analysis method. Differences between creaminess hedonic liking clusters tested by independent t test, \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$ .

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The sweet hedonic liking ratings of creaminess likers and dis-likers are shown in Figure 6.4. Interestingly, sweet hedonic liking ratings of both creaminess hedonic liking clusters decreased with increasing sucrose concentrations. However, creaminess likers had significantly higher sweet hedonic liking ratings for 180 mM, 360 mM and 720 mM sucrose compared to creaminess dis-likers ( $p < 0.01$ , Figure 6.4).



**Figure 6.4 Sweet hedonic liking ratings of creaminess likers and dis-likers**

Data shown as mean  $\pm$  SEM. Creaminess likers  $n=176$  and creaminess dis-likers  $n=211$ . Differences between creaminess hedonic liking clusters tested by independent t test, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

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## 6.4.3 Dietary intake measurements

### 6.4.3.1 Frequency of food group intake

Table 6.2 presents the median DFE scores of the 15 most and least consumed food groups obtained using the 220-item FFQ. Overall, green vegetables, oil and oil based dressing, non-starchy vegetables and apple, banana, orange were consumed once a day. The least consumed food groups were beer, soy products, sweetened cereals and sugar added to food and drink, consumed less than once a month.

**Table 6.2 Daily frequency equivalents of the 15 most and least consumed food groups**

<b>15 most consumed food groups</b>	<b>DFE score</b>
Green vegetables	1.7[1.8, 2.6]
Oil and oil based dressings	1.4[0.8, 2.3]
Other non-starchy vegetables	1.1[0.7, 1.6]
Apple, banana, orange	1.0[0.6, 1.5]
Other fruit	1.0[0.4, 1.6]
Low-fat milk	0.7[0.01, 2.8]
Wholegrain breads	0.7[0.2, 1.4]
Tea	0.7[0.1, 2.0]
Margarine	0.6[0.01, 1.90]
Sauces	0.5[0.2, 0.9]
Coffee	0.5[0.01, 1.70]
White meats	0.4[0.3, 0.7]
Sweet snack foods	0.4[0.6, 0.9]
Dark yellow vegetables	0.4[0.2, 0.8]
Egg and egg dishes	0.4[0.2, 0.5]
<b>15 least consumed food groups</b>	<b>DFE score</b>
Wholegrains	0.07 [0.00, 0.21]
Oats	0.07 [0.00, 0.36]
Sweet spreads	0.07 [0.01, 0.36]
Savoury spreads	0.07 [0.01, 0.36]
Fruit juice	0.07 [0.01, 0.15]
Wine	0.07 [0.01, 0.29]
Coconut fats	0.02 [0.01, 0.08]
Other alcoholic beverages	0.02 [0.00, 0.14]
Diet drinks	0.01 [0.00, 0.14]
Sugar added to food and drink	0.01 [0.00, 0.36]
Sweetened cereals	0.00 [0.00, 0.13]
Soy products	0.00 [0.00, 0.01]
Beer	0.00 [0.00, 0.07]

DFE: daily frequency equivalent. Food groups and daily frequency equivalent scores were derived from the 220-item food frequency questionnaire. All data reported as median [25, 75 percentiles]. <sup>a</sup> DFE score of 0—never; 0.008—less than once per month; 0.067—1–3 times per month; 0.14—once per week; 0.36—2–3 times per week; 0.71— 4–6 times per week; 1— once a day; 2.5—2–3 times per day; 4—more than four times per day. *n*=378.

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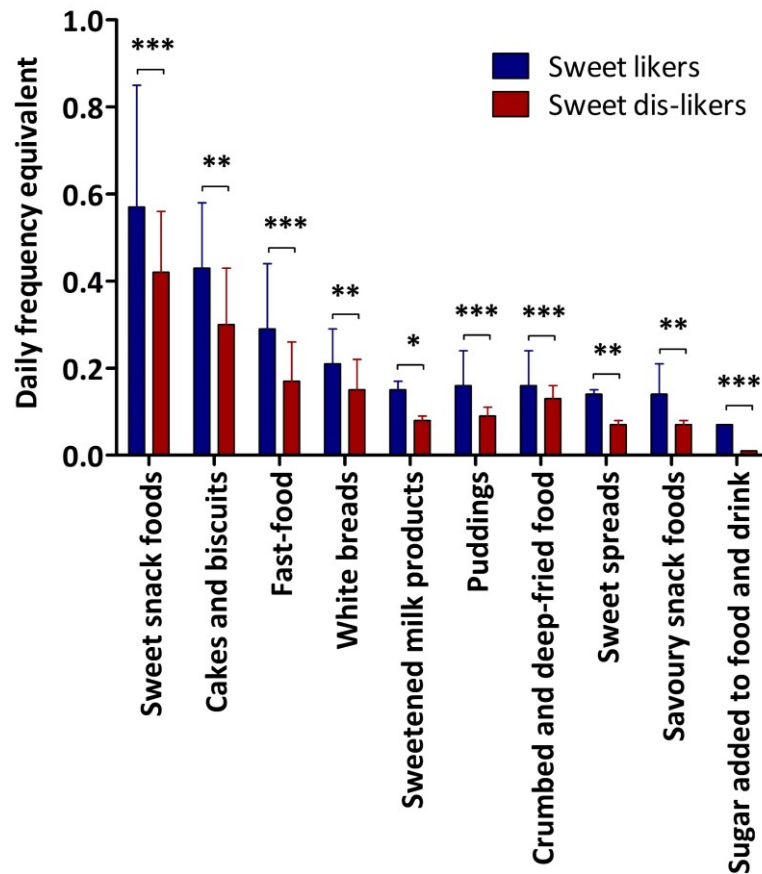
#### **6.4.3.2 Dietary patterns**

Using the PCA method and varimax rotation, four dietary patterns were identified. In-depth details of the dietary patterns can be found in Chapter 5 section 5.4.2. Briefly, the 'refined and processed' pattern was characterised by high factor loadings on crumbed and deep-fried food, fast-food, puddings, fruit drinks, soft drinks and other beverages, sweetened milk products, refined grains, starchy vegetables, white breads and sweetened cereals. The 'sweet and savoury snacking' pattern was characterised by high loadings on cakes and biscuits, sweet and savoury spreads, sweet and savoury snacks, margarine, peanut butter and peanuts, sauces, whole grain breads, cheese and crackers. The 'fruit and vegetable' pattern had high factor loadings on all fruits and vegetables (except potatoes), soy products, legumes, wholegrains and yoghurt. The 'fats and meat' pattern was characterised by high loadings on fats, all red, white and processed meats, creamy dressings, egg dishes, coconut fats, alcoholic beverages and full-fat milk and negative loadings on low-fat milk.

## 6.4.4 Patterns of hedonic liking and dietary measurements

### 6.4.4.1 Frequency of food group intake

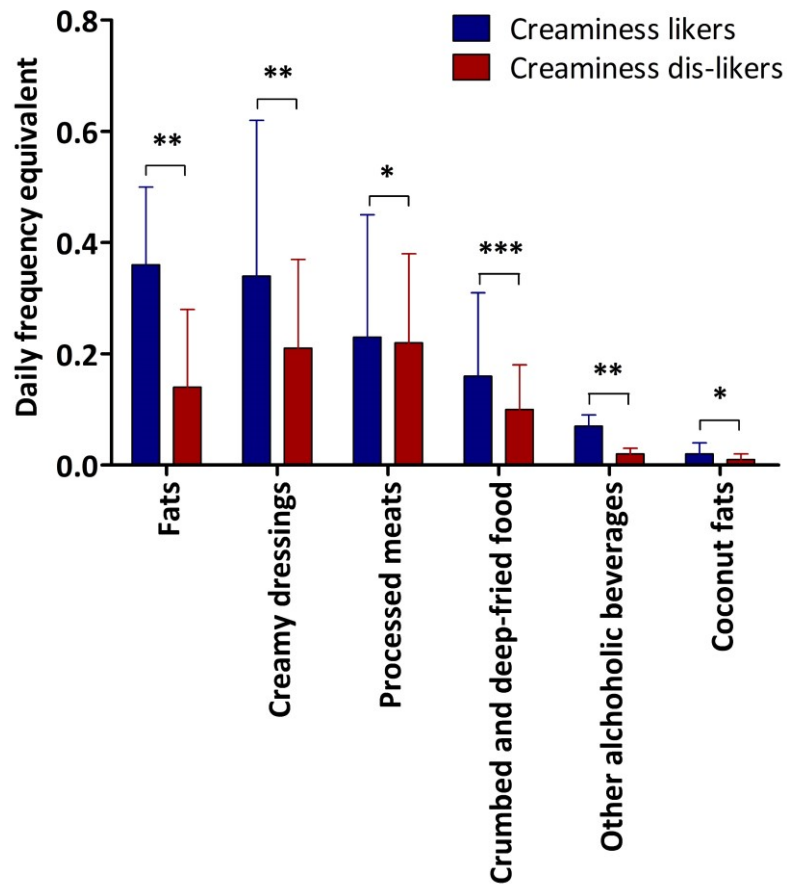
As shown in Figure 6.5, compared to sweet dis-likers, sweet likers had significantly higher frequency of intakes of sweet snack foods ( $p=0.001$ ), cakes and biscuits ( $p=0.008$ ), fast-food ( $p<0.001$ ), white breads ( $p=0.009$ ), sweetened milk products ( $p=0.022$ ), puddings ( $p<0.001$ ), crumbed and deep-fried food ( $p=0.001$ ), sweet spreads ( $p=0.015$ ), savoury snack foods ( $p=0.006$ ) and sugar added to food and drinks ( $p<0.001$ ). Furthermore, frequency of consuming green vegetables ( $p<0.04$ ) and nuts and seeds ( $p<0.05$ ) were significantly lower in sweet likers compared to sweet dis-likers.



**Figure 6.5 Frequency of food group intake of sweet likers and dis-likers**

Data reported as median (95% confidence interval for median). Sweet likers  $n=99$  and sweet dis-likers  $n=275$ . Intakes of all food groups are significantly different between sweet likers and sweet dis-likers as tested by Mann-Whitney U test, \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$ .

As seen in Figure 6.6, of the 57 food groups, creaminess likers had significantly higher frequency of intakes of fats ( $p=0.005$ ), creamy dressing ( $p=0.003$ ), processed meats ( $p=0.02$ ), crumbed and deep-fried food ( $p<0.001$ ), alcoholic beverages ( $p=0.011$ ) and coconut fats ( $p=0.017$ ) and compared to creaminess dis-likers.



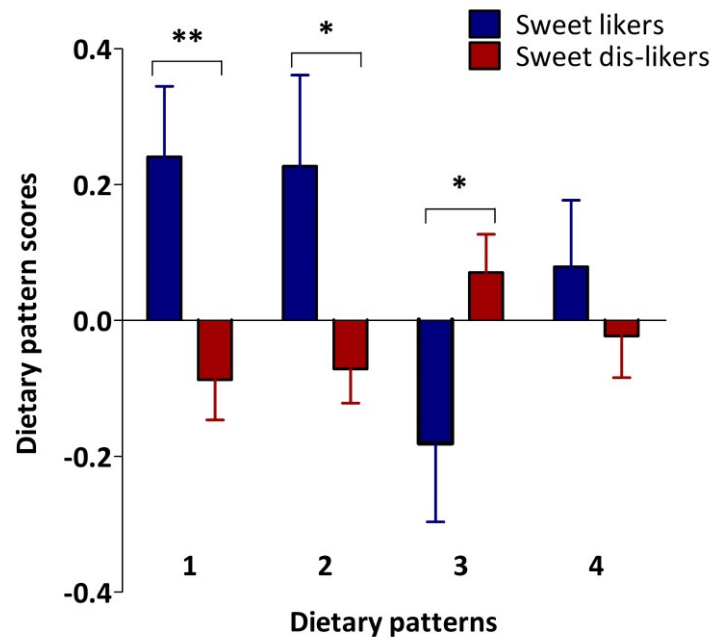
**Figure 6.6 Frequency of food group intake of creaminess likers and dis-likers**

Data reported as median (95% confidence interval for median). Creaminess likers  $n=167$  and creaminess dis-likers  $n=201$ . Intakes of all food groups are significant different between creaminess likers and creaminess dis-likers as tested by Mann-Whitney U test, \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$ .

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#### 6.4.4.2 Dietary patterns

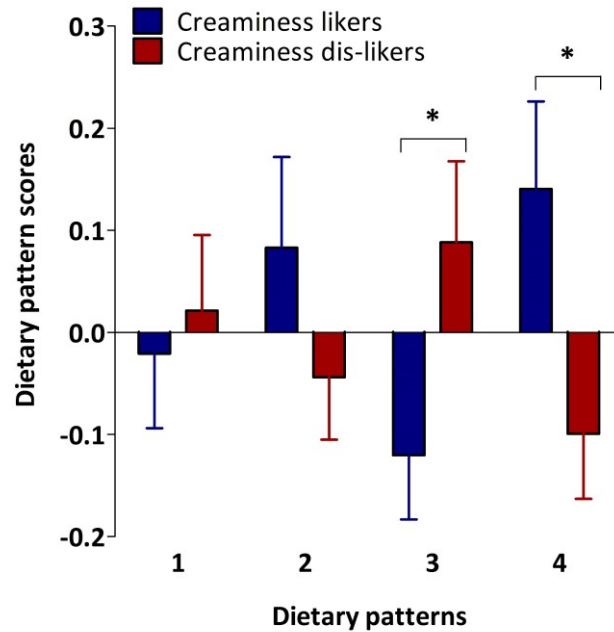
As shown in Figure 6.7, sweet likers had higher scores for the 'refined and processed' ( $p=0.007$ ) and 'sweet and savoury snacking' ( $p=0.04$ ) dietary patterns and a lower score for the 'fruit and vegetable' dietary pattern ( $p=0.04$ ) compared to sweet dis-likers.



**Figure 6.7 Dietary pattern scores of sweet likers and dis-likers**

Dietary pattern 1: 'refined and processed', 2: 'sweet and savoury snacking', 3: 'fruit and vegetable', 4: 'fats and meat'. Data reported as mean  $\pm$  SEM. Sweet likers  $n=99$ , sweet dis-likers  $n=275$ . Differences between sweet hedonic liking clusters tested by independent t test, \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$ .

As shown in Figure 6.8, creaminess likers had a lower score for the 'fruit and vegetable' dietary pattern ( $p=0.04$ ) and a higher score for the 'fats and meat' dietary pattern ( $p=0.02$ ) compared to creaminess dis-likers.



**Figure 6.8 Dietary pattern scores of creaminess likers and dis-likers**

Dietary pattern 1: 'refined and processed', 2: 'sweet and savoury snacking', 3: 'fruit and vegetable', 4: 'fats and meat'. Data reported as mean  $\pm$  SEM. Creaminess likers  $n=166$ , and creaminess dis-likers  $n=198$ . Differences between creaminess hedonic liking clusters as tested by independent t test, \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$ .

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### 6.4.5 Patterns of hedonic liking and metabolic biomarkers

Measurements of metabolic and endocrine biomarkers of sweet and creaminess hedonic liking clusters are reported in Table 6.3. Apart from sweet likers having a higher level of fasting insulin compared to sweet dis-likers ( $p<0.05$ ), no other differences in metabolic and endocrine biomarkers were observed between sweet and creaminess hedonic liking clusters.

**Table 6.3 Metabolic and endocrine biomarkers of hedonic liking clusters**

	Sweet likers	Sweet dis-likers	Creaminess likers	Creaminess dis-likers
Glucose (mmol/L)	4.66±0.04	4.68±0.02	4.71±0.03	4.65±0.03
Insulin (mU/mL)	13.86±1.04	11.54±0.47 <sup>a</sup>	12.37±0.66	12.08±0.62
HbA1c (mmol/mol)	28.67±0.33	28.52±0.23	28.47±0.28	28.68±0.25
Leptin (ng/mL)	8.71±0.001	7.41±0.001	7.94±0.001	7.59±0.001
Ghrelin (pg/mL)	45.48±3.62	48.34±2.43	46.42±2.92	48.47±2.88
Cholesterol (mmol/L)	4.62±0.09	4.58±0.05	4.55±0.07	4.60±0.06
HDL-C (mmol/L)	1.52±0.04	1.58±0.02	1.56±0.03	1.56±0.03
LDL-C (mmol/L)	2.65±0.08	2.58±0.05	2.57±0.06	2.60±0.06
Triglyceride (mmol/L)	0.99±0.05	0.96±0.04	0.94±0.03	1.00±0.06
CRP (mg/L)	0.57±0.02	0.54±0.01	3.55±1.02	3.47±1.02
IL-6 (pg/mL)	0.34±0.02	0.28±0.02	2.04±1.05	1.91±1.05
IL-10 (pg/mL)	1.08±0.04	1.01±0.03	10.47±1.10	10.96±1.07
TNF-α (pg/mL)	7.24±0.29	6.61±0.13	7.04±0.2	6.59±0.16

HbA1c: glycosylated haemoglobin, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, CRP: C-reactive protein, IL-6: interleukin-6, IL-10: interleukin-10, TNF-α: tumour necrosis factor-alpha. Data shown as mean ± SEM. Sweet likers  $n=107$ , sweet dis-likers  $n=286$ , creaminess likers  $n=176$  and creaminess dis-likers  $n=211$ . <sup>a</sup>Significantly different between sweet likers and sweet dis-likers as tested by independent t test,  $p<0.05$ .

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## 6.5 Discussion

The overall goal of the present study was to explore the links between patterns of sweet and fat (creaminess) hedonic liking, body composition, dietary intake and metabolic and endocrine biomarkers of adiposity and appetite. There were four main findings in our study. Firstly, using the hierarchical cluster analysis method we identified two very distinct patterns of sweet and creaminess hedonic liking (i.e., likers and dis-likers). Secondly, we observed higher plasma insulin levels in sweet likers compared to sweet dis-likers. However, BMI, body fat and other metabolic and endocrine biomarkers were not different between women with distinct patterns of sweet and creaminess hedonic liking. Thirdly, compared to sweet dis-likers, sweet likers had significantly higher frequency of intakes of predominantly sweet tasting food groups (e.g., sweet snack foods, cakes and biscuits). Furthermore, compared to sweet dis-likers, sweet likers had higher scores for the 'refined and processed' and 'sweet and savoury snacking' dietary patterns and a lower score for the 'fruit and vegetable' dietary pattern. Lastly, compared to creaminess dis-likers, creaminess likers had higher frequency of intakes of predominantly fatty tasting food groups (e.g., processed meats, fats), a higher score for the 'fats and meat' dietary pattern and a lower score for the 'fruit and vegetable' dietary pattern.

The overall results of our study show that in our study population, there are women with distinct patterns of sweet and creaminess hedonic liking. Further, higher sweet and fat hedonic liking is linked with increased intakes of sweet and fatty tasting food groups and higher intakes of dietary patterns such as the 'refined and processed' and 'fats and meat' patterns. Although no differences in body composition measurements were observed between the hedonic liking clusters, we could speculate that there may be adverse metabolic health consequences if consumption of dietary patterns that favour high intakes of sugar and fat are continued long-term (Te Morenga *et al.*, 2014; Riccardi *et al.*, 2004; Bray and Popkin, 1998; Willett, 2012; Malik *et al.*, 2006).

### 6.5.1 Patterns of sweet hedonic liking

We identified two distinct patterns of sweet hedonic liking. Sweet likers (27%) were characterised by a clear dislike of the two lowest sucrose concentrations and a strong like for higher concentrations. In contrast, sweet dis-likers (73%) liked the sweetness of the two lowest sucrose concentrations and disliked the sweetness of higher concentrations. A study conducted by Asao *et al.* (2015) using the hierarchical cluster analysis method identified two sweet hedonic liking groups similar to the patterns in our study; one group that liked the sweetness of low

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sucrose concentrations and another that liked the sweetness of high sucrose concentrations (Asao *et al.*, 2015). Furthermore, similar patterns of sweet hedonic liking were found by others using manual classifications (Yeomans *et al.*, 2007; Looy and Weingarten, 1991). For example, Yeomans *et al.* (2007) based on the hedonic liking ratings of four sucrose concentrations (50–830 mM) classified ‘sweet likers’ as the group with increasingly higher liking ratings with increasing sucrose concentrations (Yeomans *et al.*, 2007). In the same study, ‘sweet dis-likers’ were characterised by a decline or a rise and decline (inverted U-shape) in hedonic ratings with increasing sucrose concentrations (Yeomans *et al.*, 2007). These studies suggest that there are groups of individuals who like sweet taste at low concentrations and others who like sweet taste at higher concentrations, similar to the findings in our study.

The present study did not identify the inverted U pattern of sweet hedonic liking observed by others. The concentration at which hedonic liking is at its peak differs between studies. For example, while one study found a maximum hedonic liking at 1 M glucose (Moskowitz *et al.*, 1974), others found peak likings at 360 mM (Kim *et al.*, 2014) and >600 mM sucrose (Thompson *et al.*, 1976). These differences may be attributed to the various tastant concentration ranges used in the studies, as hedonic liking is often rated in response to the other concentrations tested. In our study, it is possible that sweet hedonic liking peaks above 720 mM sucrose. If hedonic liking of sucrose concentrations above 720 mM was assessed, we may have observed the inverted U pattern of hedonic liking.

In the present study, sweet likers perceived the sweetness of all sucrose solutions (except 20 mM) as less intense compared sweet dis-likers. This finding contrasts with previous studies where patterns of sweet hedonic liking were observed in the absence of a clear difference in sweet taste intensity (Kim *et al.*, 2014; Looy *et al.*, 1992). Although we cannot be certain if sweet hedonic liking is dependent solely on the perceived sweet taste intensity, the clear difference in intensity between sweet likers and dis-likers of our study suggests that it does influence sweet liking. This finding is supported by our previous study (Chapter 3), where we observed a positive relationship between sweet taste intensity and sweet hedonic liking at 125 mM glucose and a negative relationship at 500 mM and 1000 mM glucose, indicating a sweet taste intensity-dependent change in sweet hedonic liking (Jayasinghe *et al.*, 2017).

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## 6.5.2 Patterns of sweet hedonic liking and dietary intake

In our study, sweet likers had higher frequency of intakes of predominantly sweet tasting food groups (e.g., sweet snack foods, cakes and biscuits, white breads) and lower intakes of green vegetables, nuts and seeds compared to sweet dis-likers. In addition, sweet likers had higher intakes of the 'refined and processed' and 'sweet and savoury snacking' dietary patterns and a lower intake of the 'fruit and vegetable' dietary pattern compared to sweet dis-likers. These results suggest that women with higher sweet hedonic liking (and lower perceived sweet taste intensity) have higher frequency of intakes of refined and sweet tasting foods. Furthermore, sweet likers were more likely to consume dietary patterns high in refined, processed and snack type foods and less likely to consume a fruit and vegetable dietary pattern compared to sweet dis-likers.

To the best of our knowledge this is the first study to report differences in dietary intake (frequency of food group intake and dietary pattern) between patterns of sweet hedonic liking. One study reported that sweet likers had higher hedonic liking ratings for sweet foods such as chocolate flavoured milk and donuts and savoury foods such as cream sauce pasta, sweet and sour pork and cream cheese on bagel compared to sweet dis-likers (Kim *et al.*, 2014). Although not determined using cluster analysis, a few previous studies have shown a positive link between liking of sweet tastants in test solutions and preferences for sweet desserts (Drewnowski *et al.*, 1999), frequency of sugar and sweet food intake (Holt *et al.*, 2000), sucrose content of most preferred cereal (Mennella *et al.*, 2011) and total energy, carbohydrate and sugar intakes (Jayasinghe *et al.*, 2017).

It is interesting to note that in our study some food groups that showed differences in intakes between sweet likers and dis-likers (e.g., fast food, crumbed and deep-fried food) are high in fat content. Given that sweet likers had positive hedonic liking ratings across all fat concentrations, it not surprising to see higher intakes of fatty foods by sweet likers. Our findings suggest that sweet likers in our study could have an intrinsic hedonic liking towards fatty tasting foods. Furthermore, although no differences in BMI and body fat between sweet likers and dis-likers were observed, higher consumption of sweet tasting foods and following dietary patterns that are high in refined, processed and snack type foods may have adverse consequences on metabolic health long-term.

Although humans have an innate preference for sweet taste (Rosenstein and Oster, 1988), the present study show that there are significant inter-individual variations in sweet taste intensity and sweet hedonic liking. Variations in sweet preference and sweet food intake may be related

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to the genetic makeup of an individual. It has been suggested that variations in sweet taste perception may relate to polymorphisms of the sweet taste receptor T1R2+T1R3 (Fushan *et al.*, 2009; Chamoun *et al.*, 2016). Another study reported that approximately half of the variation in hedonic liking for sweet solutions, liking of sweet food and frequency of sweet food intake could be explained by the same genes (Keskitalo *et al.*, 2007b). Although not evaluated in the present study, the differences in sweet taste perception and dietary intake between patterns of sweet hedonic liking may be explained by variations that arise from genetic differences in the peripheral and/or central taste processing pathways (Keskitalo *et al.*, 2007b; Reed and McDaniel, 2006; Reed *et al.*, 2006; Keskitalo *et al.*, 2007a).

### **6.5.3 Patterns of fat (creaminess) hedonic liking**

The present study identified two distinct patterns of creaminess hedonic liking. Creaminess likers (45%) had positive hedonic liking ratings across all five milk samples indicating a clear liking for creaminess. Creaminess dis-likers (55%) had negative hedonic liking ratings indicating a dislike of creaminess irrespective of the fat concentration. Identifying patterns of creaminess hedonic liking is less well explored compared to sweet taste. A recent study using the hierarchical cluster analysis method identified three patterns of fat liking (Shen *et al.*, 2017). The 'non-discriminators' cluster (52%) had consistently higher liking ratings regardless of the fat content, the 'high-fat dis-likers' cluster (30%) had lower liking ratings for samples with >15% fat content and the 'high-fat likers' cluster (18%) had substantially lower liking ratings for 6% and 10% fat content (Shen *et al.*, 2017). This study suggests that there are groups of individuals with distinct hedonic liking patterns, similar to the findings of our study.

Interestingly, in the present study the perceived creaminess intensity between the creaminess hedonic liking clusters differed only at the highest fat concentration (36.9%). We could speculate that creaminess hedonic liking is influenced by a multisensory perception involving cross-modal interactions between taste, olfaction, texture and mouthfeel and not only by the perceived creaminess intensity (Frost and Janhoj, 2007). It is reported that fat taste is first detected by the olfactory perception of volatile, fat-soluble molecules through the nose followed by texture, mouthfeel and detection within the oral cavity (Montmayeur and le Coutre, 2010). Although not investigated in our study, it is possible that olfaction, mouthfeel or texture aspects of fat played a role in influencing creaminess hedonic liking, in addition to the oral detection of creaminess. As nose clips were not worn during sensory testing, we were not able to separate the taste component of fat taste from the olfactory perception. Nevertheless, in our study, there were

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distinct patterns of creaminess hedonic liking (i.e., likers and dis-likers), even without a clear difference in perceived creaminess intensity.

#### **6.5.4 Patterns of fat (creaminess) hedonic liking and dietary intake**

The present study found that compared to creaminess dis-likers, creaminess likers had higher frequency of intakes of fatty tasting food groups (e.g., processed meats, fats, creamy dressing). Creaminess likers also had a higher intake of the 'fats and meat' dietary pattern and a lower intake of the 'fruit and vegetable' dietary pattern than creaminess dis-likers. The food groups and dietary patterns that show differences between creaminess likers and dis-likers are high in fat, low in carbohydrate and predominantly fatty tasting foods. Given that creaminess likers disliked higher concentrations of sucrose, it is not surprising to see that the differences in intakes between creaminess likers and dis-likers were for predominantly fatty tasting foods. Only a handful of studies have assessed the link between fat hedonic liking using chemosensory tests and food intake. While one study found higher dairy intakes in high-fat likers compared to high-fat dis-likers (Shen *et al.*, 2017), others show no relationship between hedonic liking of fat taste and fat intake (Mela and Sacchetti, 1991; Shen *et al.*, 2017). However, the findings of our study show clearly that higher hedonic liking for fat taste is linked with increased intakes of fatty tasting food groups and the 'fats and meat' dietary pattern and a lower intake of the 'fruit and vegetable' dietary pattern.

Interestingly, some studies have shown that differences in fat taste perception and preferences for fatty tasting foods can be explained by genetic variations. For example, one study reported that in African Americans populations, polymorphisms of the fatty acid receptor CD36 were linked with oral fat perception (i.e., perceived fat taste) and liking of added fats and oils (Keller *et al.*, 2012). Another study reported a link between characteristics of saliva (lipolysis, lipocalin and flow) and fat taste perception (Neyraud *et al.*, 2012). Although not assessed in the present study, genetic variations (i.e., receptor polymorphisms) and characteristics of saliva may explain some differences in taste perception and dietary intake observed between creaminess likers and dis-likers.

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### **6.5.5 Patterns of hedonic liking, body composition and metabolic and endocrine biomarkers**

We observed no significant differences in BMI or body fat between patterns of sweet and creaminess hedonic liking. Furthermore, there were similar numbers of women from each ethnic group and BMI and body fat group in each sweet and creaminess hedonic liking cluster. One previous study reported that even though hedonic liking of sweet and creamy solutions did not predict BMI or adiposity at baseline, a stronger hedonic liking for these solutions was associated with weight gain at a five-year follow-up period (Salbe *et al.*, 2004). This suggests that preferences for sweet and creaminess tastes may be associated with the development of obesity possibly driven by preferences for highly palatable and energy-dense food items (Salbe *et al.*, 2004).

In the present study, sweet likers had higher levels of fasting insulin compared to sweet dislikers. We could speculate that in sweet likers, increased intakes of predominantly sweet tasting foods and following dietary patterns that favour refined and processed foods (high in starch and sugar) could promote hyper-insulin secretion (Daly *et al.*, 1998). Alternatively, hyper-insulin secretion could stimulate cravings for sweet food, as observed in situations of hyper-insulineamia associated with gestational diabetes that is known to increase adiposity in the long term (Belzer *et al.*, 2010). Given that generally healthy participants were recruited in the present study, it was not surprising to find no other significant differences in metabolic and endocrine biomarkers between patterns of sweet and creaminess hedonic liking. Although we did not observe any differences in body composition and metabolic biomarkers between the hedonic liking clusters, prolonged practice of unhealthy dietary intake driven by taste preferences may increase susceptibility towards weight gain. Therefore, future studies should investigate the long-term effect of sweet and creaminess hedonic liking on body composition and metabolic biomarkers in a prospective study.

### **6.5.6 Strengths and limitations**

The present study has several strengths. Firstly, sweet and creaminess intensity and hedonic liking was assessed using the gLMS, which is considered the most reliable and valid method of capturing subjective experiences and comparing taste perceptions between groups (Snyder *et al.*, 2006; Bartoshuk *et al.*, 2006). Secondly, characterising an individual's sweet and fat taste hedonic liking based on their liking across multiple concentrations is superior to an approach that uses a single concentration. In addition, the hierarchical cluster analysis provided an unbiased method of grouping individuals according to patterns of hedonic liking. Thirdly, in

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addition to assessing the frequency of food group intake, differences in dietary patterns between hedonic liking clusters were also evaluated. Traditionally, nutrition research has focused on single nutrients or specific foods to assess the link between diet and nutrition-related health outcomes. However, individuals do not consume nutrients or foods in isolation and dietary patterns provide a better understanding of the overall diet as it includes food groups commonly consumed together (Cespedes and Hu, 2015). The observed differences in the dietary patterns between the sweet and creaminess hedonic liking patterns of the present study further strengthens the need for investigating dietary patterns in relation to taste.

There are several limitations of the present study that should be addressed in future research. Firstly, cluster analysis was performed for sweet and creaminess perception separately. As mixtures of sweet and fat may exhibit hedonic synergy (Drewnowski and Greenwood, 1983), future studies should assess the perception of sweet and fat tastants combined to identify varying patterns of liking. Secondly, dietary intake was assessed using frequency of food group intake and dietary patterns. Therefore, the next step would be to assess whether total energy, macronutrients intakes and eating behaviour factors are linked with sweet and creaminess hedonic liking phenotypes, to further our understanding of the link between hedonic liking and other dietary parameters. Thirdly, the participants of our study are a convenience sample from three ethnic groups in New Zealand. Although participants with a wide range of BMI and body fat profiles were recruited to the study, possibly due to the exclusion criteria, the study population were generally healthy (indicated by metabolic and endocrine biomarkers). Future studies should assess links between patterns of sweet and creaminess hedonic liking and dietary intake in other population groups such as men, different age, BMI and ethnic groups and between individuals with much more varied eating behaviour and food intakes. As different population groups may have different dietary intakes and dietary patterns associated with their taste patterns, knowledge about this relationship can be used to create tailored dietary interventions and dietary advice. Future studies should also assess whether genetic variations (i.e., receptor polymorphisms) and characteristics of saliva differ between sweet and creaminess likers and dis-likers, to further our understanding of the genetic and biological mechanisms that drive differences in taste perception and dietary intakes. Lastly, the links found between sweet and creaminess hedonic liking patterns and dietary intake cannot be generalised unless similar patterns are found in other populations.

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## 6.6 Conclusions

The findings of this study show for the first time a clear link between distinct patterns of sweet and fat hedonic liking and dietary intake parameters. More specifically, we found that women with higher sweet hedonic liking had higher intakes of predominantly sweet tasting food groups and higher intakes of the 'refined and processed' dietary pattern. Furthermore, women with higher fat hedonic liking had higher intakes of fatty tasting food groups and the 'fats and meat' dietary pattern. We did not find differences in body composition between women with different patterns of sweet and fat hedonic liking. However, we can speculate that higher levels of fasting insulin in sweet likers reflect a mechanistic pathway that may lead to increased adiposity in the long-term as a result of consuming energy-dense diets (Thompson *et al.*, 2007; Saltiel, 2012; Mendoza *et al.*, 2007).

Although humans have an innate preference for sweet taste, it is suggested that hedonic liking for sweet taste is driven by prior exposure to food environments that favour foods rich in sugar, especially during childhood (Ventura and Mennella, 2011; Mennella, 2014). Therefore, the findings of this study highlight the need to intervene at an early age to positively influence learned taste preferences by reducing the exposure to high concentrations of sugar and fat and offering healthy foods (e.g., fruits and vegetables) (Ventura and Worobey, 2013; Mennella, 2014). Furthermore, the current obesogenic food environment of easily accessible, energy-dense foods that are rich in sugar and fat exploit people's biological, psychological and economic vulnerabilities, thus making it easier to access unhealthy foods (Vandevijvere and Swinburn, 2014; Swinburn *et al.*, 2011). The link between higher hedonic liking and intakes of sugar- and fat-rich dietary patterns found in our study suggest that higher hedonic liking for sweet and fat tastes could be an important driver reinforcing the link between palatability and the intake of energy-dense foods, probably enabled by the unhealthy food environment (Mendoza *et al.*, 2007; Drewnowski, 1997b; Drewnowski, 1995; Drewnowski and Greenwood, 1983). These findings support the need to improve the quality of diet by creating a healthier food environment, one that consists of more affordable healthy food options and lowers the exposure to foods with high concentrations of sugar and fat (Spiteri and Soler, 2018; Yeung *et al.*, 2017; Gorton *et al.*, 2010).

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## Chapter 7

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### *Final Discussion*

#### **7.1 Overview**

Foods high in sugar and fat are highly palatable and reinforcing in comparison to foods with low sugar and fat (Drewnowski and Greenwood, 1983; Tellez *et al.*, 2016). The positive hedonic liking associated with sweet and fat tastes may provide an explanation for why these dietary components are harder to resist (Drewnowski, 1995). Furthermore, people learn to associate the sensory properties of energy-dense sweet and fatty foods with rewarding post-ingestive metabolic effects (Drewnowski, 1995). Consequently, sensory properties give rise to expectations about foods and they become signals which drive subsequent food selection (Stubbs and Whybrow, 2004). In addition, inter-individual differences of how sweet and fat tastes are detected, perceived and liked can influence food choice and impact long-term health.

The specific objectives of this thesis were to:

1. Explore the relationship between four commonly used measurements of sweet taste perception (detection threshold, recognition threshold, sweet taste intensity, sweet hedonic liking) and investigate which measurements of sweet taste perception are linked with sweet food intake. (Chapter 3)
2. Evaluate differences in sweet taste and fat (creaminess) perception across different ethnic groups with known differences in metabolic disease and obesity risk (NZE, Māori, Pacific) and across well-defined body composition groups (BMI, body fat). Furthermore, to explore the relationship between sweet taste and fat (creaminess) perception and key metabolic and endocrine biomarkers of adiposity and appetite. (Chapter 4).
3. Identify dietary patterns in women from different ethnic groups with known differences in metabolic disease and obesity risk and to investigate the link between these dietary patterns, body composition, macronutrient intakes and metabolic and endocrine biomarkers of adiposity and appetite. (Chapter 5)
4. Identify and characterise patterns of sweet and fat (creaminess) hedonic liking and to investigate whether body composition, dietary intake and metabolic and endocrine biomarkers of adiposity and appetite differ between women with different patterns of hedonic liking. (Chapter 6)

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The present thesis contains four experimental chapters outlining the main findings of the two multi-disciplinary cross-sectional studies. The first cross-sectional study was conducted to achieve objective one, where the link between four different measurements of sweet taste perception and sweet food intake were assessed in a group of 44 NZE women (Chapter 3). Using data from the second cross-sectional study, objectives two to four were investigated in a sample of 408 NZE, Māori and Pacific women (Chapters 4–6).

## **7.2 Main findings**

The main findings of the four experimental chapters of this thesis are discussed in the following four subsections.

### **7.2.1 Sweet taste perception measurements and sweet food intake**

Taste perception is experienced when tastants from foods and beverages bind to taste receptors in the gustatory system (Chandrashekar *et al.*, 2006). The activation of taste receptors initiates a downstream signalling cascade which relays information about the quality and strength of the taste to the brain (Simon *et al.*, 2006). As there is no single ubiquitously used method of assessing taste perception (Webb *et al.*, 2015), four commonly used psychophysical measurements of sweet taste perception were chosen for the study in Chapter 3. Each sweet taste measurement was chosen as it characterises a distinct component of taste perception (Lim *et al.*, 2009; Bartoshuk, 1978). Detection and recognition thresholds provide estimates of the lowest sweet tastant (glucose) concentration that can be detected or recognised, while sweet taste intensity measures the magnitude of sweetness produced by suprathreshold glucose concentrations (Lawless and Heymann, 1999). The fourth measure, sweet hedonic liking, provides a measurement of preference or innate liking of suprathreshold glucose concentrations (Lim *et al.*, 2009). One of the aims of Chapter 3 was to evaluate and compare the above four measurements of sweet taste perception. The large inter-individual variations in all four measures of sweet taste confirmed that the glucose concentrations used in the study evoked different levels of detection and recognition thresholds, sweet taste intensities and sweet hedonic likings. Furthermore, the intraclass correlation coefficient statistic indicated good between-session correlations for all four psychophysical measurements, strengthening the taste perception data used in the study.

One of the novel aspects of the research study in Chapter 3 was the description of glucose thresholds for each participant using individual sweet taste threshold detection curves. The detection of sweet taste of all participants increased with increasing glucose concentrations.

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However, each participant had a distinct sweet detection curve with a distinct rate of sweet taste detection, which indicated clear inter-individual variations in sweet taste sensitivity. Interestingly, it was clear that there were no correlations between glucose thresholds and sweet taste intensity or sweet hedonic liking at any suprathreshold glucose concentration. It is generally accepted that individuals with a lower detection or recognition threshold are more sensitive to the tastant than those with a higher threshold (Webb *et al.*, 2015). One might speculate that individuals who are able to detect or recognise glucose at a lower concentration would consequently perceive a greater sweet intensity when presented with suprathreshold concentrations and/or like sweet tastants at a lower concentration. Similar to previous research, our data clearly showed that the lowest concentration of a sweet tastant detected or recognised is not linked with the magnitude of sweetness experienced or how different sweet tastant concentrations are liked (Mattes, 1985; Webb *et al.*, 2015).

A further important finding in Chapter 3 was the dose-dependent change in the relationship between sweet taste intensity and sweet hedonic liking. This change in the relationship clearly indicates the presence of a biological relationship between the perceived taste intensity and hedonic liking. As discussed in Chapter 3, the low concentration of glucose was liked by women who were sensitive enough to perceive the sweetness. Furthermore, the high concentrations of glucose were disliked by women who perceive it as more sweet compared to women who perceive it as less sweet. These findings suggest at least for sweet taste, that hedonic liking is related to the magnitude of sweetness experienced. Furthermore, the finding that participants generally disliked the two highest concentrations has implications for our food environment, because these levels of sugars or other sweeteners are commonly found in sweet beverages. Our study suggests that there is ample scope to reduce the sugar content in sugar-sweetened beverages but still maintain hedonic

The second aim of the research study in Chapter 3 was to explore the relationship between different psychophysical measurements of sweet taste perception and sweet food intake. Three dietary measurements were used to capture different aspects of sweet food intake and liking. The weighed food record assessed energy and macronutrient intakes, the sweet food focused FFQ assessed habitual intakes of sweet tasting foods and the beverage liking questionnaire assessed liking of sweet beverages. As seen in Chapter 3, despite the significant inter-individual variations, glucose detection and recognition thresholds did not correlate with any of the dietary measurements. Our findings are supported by the notion that concentrations used to assess taste thresholds are below the tastant concentrations commonly found in foods and beverages,

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thus taste thresholds does not appear to provide a strong sensory basis for a link with dietary intake (Bartoshuk *et al.*, 2006).

One of the most important findings of Chapter 3 was that women who perceived glucose solutions as more sweet had lower energy, carbohydrate and sugar intakes, had a lower frequency of sweet food consumption and liked sweet beverages less than women who perceived the glucose solutions as less sweet. Furthermore, women with higher sweet hedonic liking had higher intakes of total energy, carbohydrate and sugar (total sugar, fructose, glucose). One of the strengths of Chapter 3 was that energy and macronutrient intakes were determined using weighed food records, the gold standard method of assessing nutrient intakes (Biro *et al.*, 2002). Food records provided an accurate and a superior method of assessing nutrient intakes in comparison to the FFQ methodology which is most commonly used in taste perception research. The study in Chapter 3 showed for the first time a clear link between a lower perceived sweet taste intensity and higher sweet hedonic liking and increased intakes of total energy, carbohydrate and sugar. These findings also support the notion that suprathreshold taste measurements are more closely related to dietary intake. The findings of Chapter 3 have clear implications for metabolic health and obesity development, and warrant further investigation as described in Chapters 4, 5 and 6 of this thesis.

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### 7.2.2 Sweet taste and fat (creaminess) perception, body composition and metabolic biomarkers

In the experimental study in Chapter 4, sweet taste and fat (creaminess) perception was assessed by rating the perceived taste intensity and hedonic liking. The findings of Chapter 3 showed that sweet taste thresholds were not associated with dietary intake while suprathreshold measurements of taste intensity and hedonic liking showed clear links with dietary measurements. Therefore, suprathreshold taste measurements were chosen for the study in Chapter 4 as concentrations above thresholds produce a wider taste sensory experience that is more suitable to assess links with dietary intake and body composition (Bartoshuk *et al.*, 2006; Low *et al.*, 2016). Fat taste perception was measured by rating the creaminess intensity and creaminess hedonic liking of five milk samples with varying concentrations of fat. Given that milk samples were used as the fat tastant, assessing the ‘creaminess’ provided an overall sense of the different sensations experienced when tasting milk (i.e., taste, mouthfeel) (Frost and Janhøj, 2007). In addition, to emulate normal eating experiences nose clips were not worn during any of the taste perception measurements. As adult women from different ethnic groups and different body composition groups were recruited for the EXPLORE study, to enable valid between-group comparisons the gLMS was used for rating taste intensity and hedonic liking (Bartoshuk *et al.*, 2003; Bartoshuk *et al.*, 2005).

One of the aims of Chapter 4 was to explore differences in sweet taste and fat (creaminess) perception between ethnic groups with known differences in metabolic disease and obesity risk profiles (NZE, Māori, Pacific). New Zealand is the third most obese country in the OECD (Organisation for Economic Co-operation and Development, 2017) with obesity affecting Māori and Pacific ethnic groups disproportionately compared to the general population (Ministry of Health, 2017). Therefore, it is important to assess whether differences in taste perception between ethnic groups may provide an explanation for their dietary choice. The overall results of Chapter 4 showed no robust differences in taste intensity or hedonic liking of sweet and fat tastes between ethnic groups with different metabolic disease and obesity risk profiles. The results suggested that women from all ethnic groups perceived the same level of sweet and fat taste intensity and have similar sweet and fat taste hedonic likings. As no clear differences in taste perception between ethnic groups were found in our study population, the pooled sample of all three ethnic groups was used for subsequent data analysis in Chapters 4–6.

The second aim of the study described in Chapter 4 was to explore differences in sweet taste and fat (creaminess) perception between different BMI and body fat groups. BMI and body fat

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groups were well-defined and differed significantly in terms of their body composition and physiology (i.e., metabolic biomarkers and endocrine regulators). Our findings showed that all groups perceived the same level of sweet and fat taste intensity and have similar sweet and fat taste hedonic likings. These findings support previous research that showed no direct link between sweet and fat taste perception and obesity (Pepino *et al.*, 2010; Low *et al.*, 2016; Salbe *et al.*, 2004; Pepino and Mennella, 2014).

There were no strong direct relationships between sweet taste and fat (creaminess) perception and any metabolic, endocrine or inflammatory biomarkers in Chapter 4. Participants of the EXPLORE study were in good general health (i.e., all metabolic biomarkers were within the normal healthy ranges) based on the experimental design and exclusion criteria of the study (see section 4.3.1). It is likely that the good health status may have contributed to the lack of associations between taste perception measurements, body composition and metabolic biomarkers, since changes in sweet taste perception measurements were observed in individuals with altered metabolic statuses (e.g., diabetes, following gastric bypass surgery) (Wasalathanthri *et al.*, 2014; Tepper and Seldner, 1999; Pepino *et al.*, 2014; Nance *et al.*, 2018).

In our study, the liquid tastants used to assess sweet taste and fat (creaminess) perception (i.e., sucrose dissolved in water and milk samples) were developed to obtain valid psychophysical evaluations of taste measurements similar to protocols used in previous publications (Holt *et al.*, 2000; Salbe *et al.*, 2004). Liquid tastants may not accurately reflect real-world eating experiences and may not be directly comparable to the sensory properties of solid foods or beverages. The liquid tastants may have influenced the participant's taste rating responses and may have masked potential relationships between taste perception, body composition and metabolic biomarkers. However, the findings of Chapter 4 showed very clearly that in our generally healthy study population, sweet and fat taste intensity and/or hedonic liking do not explain differences in metabolic disease risk or BMI and body fat profiles. Therefore, we could speculate that the key link between taste perception and metabolic health is through diet. Therefore, in Chapter 5 the relationship between dietary patterns and parameters of metabolic health was assessed.

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### 7.2.3 Dietary patterns, macronutrient intakes, body composition and metabolic biomarkers

In Chapter 5, using the PCA four distinct dietary patterns were identified; refined and processed, sweet and savoury snacking, fruit and vegetable, and fats and meat patterns. All dietary patterns were characterised by high factor loadings of distinct food groups that represented the dietary pattern and showed similarities with dietary patterns published by others (Naja *et al.*, 2012; Paradis *et al.*, 2009; Fonseca *et al.*, 2012). By extracting dietary patterns, we simplified a large quantity of dietary data obtained from the 220-item FFQ to a smaller set of factors (i.e., dietary patterns) that were easier to interpret and more useful to assess links with metabolic health markers.

The aim of Chapter 5 was to investigate the relationships between dietary patterns, body composition, macronutrient intakes and metabolic and endocrine biomarkers. Dietary pattern scores were categorised into tertiles to differentiate between women with low intakes (lower scores) from those with high intakes (higher scores). One important finding was that women with higher scores for the 'refined and processed' pattern had higher body composition and anthropometric measurements (e.g., BMI, body fat, WC) and higher intakes of energy and carbohydrates (starch, total sugar, sucrose) compared to women with lower scores. Furthermore, women with higher scores for the 'refined and processed' pattern had higher circulating concentrations of insulin and leptin and lower levels of ghrelin and HDL-C compared to those with lower scores. Taken together, these data suggest that higher intakes of the 'refined and processed' dietary pattern (i.e., higher energy, starch and sugar intakes) may drive hyper-insulin secretion, leading to increased deposition of adipose tissue and hyper-leptinaemia, describing the pathogenesis of obesity (Schwartz *et al.*, 2017; Saltiel, 2012). Additionally, changes in the action of endocrine regulators including insulin, leptin and ghrelin disturbed appetite regulation in the obese state, rendering a stable body weight or weight loss difficult to achieve. We could speculate that in this setting, the regulation of energy balance is biased towards protection against weight loss, further fat accumulation and disease progression (Schwartz *et al.*, 2017; Saltiel, 2012).

As discussed in Chapter 5, there were no significant differences in body composition (after adjusting for energy intake) or metabolic biomarkers between the tertiles of other dietary patterns. The lack of associations between dietary patterns and health outcomes may be attributed to the generally good health status of the study participants and/or the lower variance in food intake explained by these dietary patterns. It is possible that dietary patterns

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that do not explain much of the total variance in food intake also do not explain much of the variance in body composition or metabolic health biomarkers (Schulze *et al.*, 2007). As suggested by Randall *et al.* (1990), a link between dietary patterns and disease risk is most probably identifiable among those patterns contributing most to the variance in food intake (Randall *et al.*, 1990).

In Chapter 5 we observed significant differences in dietary patterns between women with markedly different metabolic disease and obesity risk profiles. We found significantly more Māori (51%) and Pacific (68%) women in tertile 3 (higher intakes) of the ‘refined and processed’ pattern. Additionally, there were significantly more NZE (40%) women in tertile 3 of the ‘sweet and savoury snacking’ pattern. As described in Chapter 5, the metabolic and endocrine consequences of following the ‘refined and processed’ pattern (i.e., refined carbohydrates and added sugar) may be a driver of higher adiposity and increased metabolic disease risk observed in Māori and Pacific women (Thompson *et al.*, 2007; Saltiel; Schwartz *et al.*, 2017; New Zealand Medical Association, 2014). One limitation of Chapter 5 was the uneven sample sizes between the different ethnic groups, where sample sizes of Māori and Pacific women were smaller than NZE women. Nonetheless, our findings suggest a mechanistic pathway by which increased consumption of the ‘refined and processed’ dietary pattern could lead to adverse metabolic health consequences, particularly in ethnic groups with higher metabolic disease and obesity risk. As discussed in Chapter 4, all ethnic groups had similar sweet taste and fat (creaminess) perception. Therefore, we could speculate that differences in dietary patterns between the ethnic groups are driven by broader determinants of health (e.g., socio-economic factors) that influence the types of food chosen by different ethnic groups (Southwick *et al.*, 2012; Ministry of Health, 2015c; Reremoana *et al.*, 2015). Therefore, future studies should investigate the link between dietary patterns and health outcomes in each ethnic group separately taking into account the broader determinants of health. Furthermore, different ethnic groups will require specific approaches; future studies should include culturally specific approaches to establish prevention strategies that specifically address these health inequities.

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#### **7.2.4 Patterns of sweet and fat (creaminess) hedonic liking, body composition, dietary intake and metabolic biomarkers**

The first aim of the research study in Chapter 6 was to identify and characterise patterns of sweet and fat (creaminess) hedonic liking using the hierarchical cluster analysis method. Two distinct clusters with significantly different sweet and creaminess hedonic liking patterns were identified. Patterns of hedonic liking found in Chapter 6 were observed in other studies strengthening the data obtained from the cluster analysis method (Asao *et al.*, 2015; Looy and Weingarten, 1991; Shen *et al.*, 2017). One strength of Chapter 6 was that participants were grouped according to their hedonic liking ratings across all five tastant concentrations instead of using the hedonic liking ratings of one concentration. For this purpose, the hierarchical cluster analysis method was a suitable data-driven statistical approach for determining patterns and grouping participants into non-overlapping groups. One limitation of this approach was that sweet and creaminess hedonic liking clusters were determined for each taste individually. This was done because intensity and hedonic liking ratings were conducted for sweet and fat tastes separately instead of using a combination of sweet and fat tastants. Another limitation was that cluster analysis is dependent on the particular data being used and different taste clusters may be found in other populations, making direct comparisons between studies difficult.

The second aim of Chapter 6 was to explore whether body composition, dietary intake and metabolic biomarkers differ between patterns of sweet and creaminess hedonic liking. We found clear differences in the frequency of food group intakes and dietary patterns between sweet and creaminess likers and dis-likers. Sweet likers had a higher frequency of intakes of predominantly sweet tasting food groups (e.g., sweetened milk products, cakes and biscuits) than sweet dis-likers. One of the important findings of Chapter 6 was that sweet likers had higher intakes of the 'refined and processed' and 'sweet and savoury snacking' dietary patterns and had a lower intake of the healthy 'fruit and vegetable' dietary pattern compared to sweet dis-likers. Although not determined using cluster analysis, a few previous studies have also shown a positive link between liking of sweet solutions and preferences for and intakes of sweet foods, supporting the findings of our study (Drewnowski *et al.*, 1999; Holt *et al.*, 2000; Mennella *et al.*, 2011). Chapter 6 shows for the first time that women with a higher hedonic liking for sweet taste were more likely to consume dietary patterns characterised by refined, processed and snack type foods and were less likely to consume a healthy fruit and vegetable diet.

Another interesting finding in Chapter 6 was that compared to creaminess dis-likers, creaminess likers had higher frequency of intakes of fatty tasting food groups (e.g., fats, crumbed and deep-

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fried food). In addition, creaminess likers had a higher intake of the 'fats and meat' pattern and a lower intake of the healthy 'fruit and vegetable' pattern. These findings suggest that women with a higher hedonic liking for fat taste, consume foods that are predominantly fatty tasting and high in fat and consume less of a healthy diet consisting of fruits and vegetables.

The findings in Chapter 6 showed that sweet likers had higher levels of fasting insulin levels compared to sweet dis-likers. We could speculate that increased intakes of predominantly sweet tasting foods and higher intakes of the 'refined and processed' dietary pattern could promote hyper-insulin secretion (Daly *et al.*, 1998). As discussed in Chapter 5, higher intakes of the 'refined and processed' dietary pattern highlighted a pathway to obesity described by higher body composition measurements and changes in endocrine regulators of adiposity and appetite. In Chapter 6, although BMI and body fat did not differ between sweet likes and dis-likers, we could speculate that prolonged intakes of the 'refined and processed' dietary pattern by sweet likers may increase their risk of weight gain and adverse health outcomes over time.

The findings of Chapter 6 have implications for food choice and eating behaviour. Higher sweet and fat taste hedonics seem to play a significant role in the increased intakes of food groups and dietary patterns that favour sugar- and fat-rich foods. These findings are especially important in the current 'obesogenic' food environment where sweet- and fat-rich foods are easily accessible. Higher hedonic liking for sweet and fat tastes can promote higher intakes of energy-dense, nutrient-poor, refined and processed foods high in sugar and fat, thereby increase passive over-consumption of energy. Furthermore, by assessing whether dietary patterns differed between patterns of sweet and fat taste hedonic liking, we gained a better understanding of the combinations of foods consumed together. It has been highlighted previously that dietary patterns have the advantage of being amenable to public health recommendations, as the focus is on the entire diet rather than one food group or nutrient (Hu, 2002). Therefore, understanding the relationship between patterns of sweet and fat hedonic liking and dietary patterns may help develop dietary interventions to reduce the caloric over-consumption from sweet and fatty tasting foods associated with the 'refined and processed' and 'fats and meat' dietary patterns.

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### 7.3 Limitations of the studies and future research recommendations

The findings and limitations of the four experimental chapters of this thesis highlight important recommendations for future studies.

One important methodological aspect of this thesis was that both research studies were cross-sectional in design. Cross-sectional studies measure both the exposure and outcome at a specific point in time within a population of interest (Pandis, 2014). Cross-sectional studies can be descriptive (measure prevalence of disease) or analytical (data are used to examine relationships between measured variables). There are many advantages of the cross-sectional study design. For example, they are easier to conduct and less expensive than intervention studies and the study design allows examining associations between multiple variables which is useful to determine burden of disease (Levin, 2006; Sedgwick, 2014). More importantly, the outcomes can be used to generate new hypotheses and to design more rigorous in-depth longitudinal studies. Disadvantages include the requirement of a large sample size to accurately assess relationships, the temporality of single assessments, the influence of confounding factors that can mask true associations between exposure and outcome, and potential selection bias where the study sample is systematically different from the population of interest (Sedgwick, 2014; Pandis, 2014). Furthermore, cross-sectional studies can only ascertain a link or a relationship and no cause and effect can be established due to the bidirectional nature of some associations (Levin, 2006). For example, the significant links between sweet taste and fat (creaminess) perception and dietary intake reported in Chapters 3 and 6 only show relationships. Future dietary intervention studies (e.g., reduced sweet food or reduced refined and processed food) should be conducted to test whether sweet and fat taste intensity and hedonic liking can be altered in response to dietary changes. In addition, further work is required to assess whether the change in taste parameters translate to changes in the type and amount of food they choose and consume. In Chapter 5, we can only establish a link between higher intakes of the 'refined and processed' dietary pattern and adverse metabolic health outcomes. Future dietary intervention studies should confirm whether a diet lower in refined and processed foods would result in changes in body composition measurements and metabolic health biomarkers.

Although this thesis has many important findings, both cross-sectional studies were conducted in women and the findings cannot be generalised to men. Women were chosen for both studies due to the increased rates of obesity observed in New Zealand women and due to the long-term health impact of increased adiposity in women of child-bearing age (Marchi *et al.*, 2015; Crane

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*et al.*, 2009; Ministry of Health, 2017). Further research should be done to investigate the relationships between taste perception, dietary intake and obesity in larger trials and in other populations such as men, different BMI and age groups, in population groups with different levels of obesity risk and people with healthy and unhealthy eating habits.

As discussed in Chapter 4, sweet taste and fat (creaminess) perception was not different between ethnic groups. Due to the significant challenges encountered during recruitment, the sample sizes of Māori and Pacific women were significantly smaller than NZE women. Therefore, future studies with larger sample sizes should be conducted to obtain more statistical power to detect small differences in taste parameters between the ethnic groups. The findings in Chapter 5 clearly showed differences in dietary patterns between women with different metabolic disease and obesity risk profiles (NZE, Māori, Pacific). This demonstrates the need to explore the link between dietary patterns and nutrition-related health outcomes in each ethnic group separately. Further research is also needed to tackle barriers and enablers to engaging in healthy lifestyle practices, especially those impacting on populations at greatest risk of developing obesity.

One of the main objective of this thesis was to assess the perception of taste. However, to emulate normal eating circumstances nose clips were not worn during the taste perception measurements. Therefore, it is possible that olfaction may have influenced the participant's creaminess responses in Chapters 4 and 6. Furthermore, all tastants used in the experimental studies were liquid and may not resemble foods and beverages consumed in real-world eating circumstances. Future studies should assess gustation, olfaction and textural properties separately using standardised methods of testing and real-food matrices to provide a better comparison with real-life eating experiences. In Chapter 6, patterns of hedonic liking were established for sweet and fat tastes separately. Future research should assess the perception of the sweet and fat tastants combined to identify patterns of sweet and fat taste liking. In addition, how the combination of sweet and fat tastes relate to dietary intake and body composition should also be assessed. Future studies should also explore the relationships between the perception of other tastes (e.g., salt, bitter, umami), body composition, dietary measurements and metabolic biomarkers to further our understanding of the link between taste, dietary intake and obesity.

We did not observe differences in sweet taste and fat (creaminess) perception between the three ethnic groups or across different body composition groups (Chapter 4). However, many studies have shown links between genetic aspects of taste perception, dietary intake and

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obesity (Keskitalo *et al.*, 2007a; Reed *et al.*, 2006; Chamoun *et al.*, 2016). Future research should investigate differences of known genetic variations in sweet and fat taste receptors (e.g., polymorphisms in genes) between different groups to further our understanding of the biological link between taste, dietary intake and obesity.

It has been highlighted throughout this thesis that the current 'obesogenic' food environment is a significant driver of obesity and metabolic disease risk (Vandevijvere and Swinburn, 2014; Brinsden *et al.*, 2013). As discussed in Chapter 5, higher intakes of the 'refined and processed' dietary pattern clearly highlighted a pathway to obesity indicated by the adverse metabolic health outcomes. In addition, a clear link between sweet taste and fat (creaminess) perception and dietary intake was observed in Chapter 6, where sweet likers and creaminess likers followed the 'refined and processed' and the 'fats and meat' dietary pattern respectively. Interestingly, recent research shows that the gustatory system is less able to detect the nutrient content of highly processed foods compared to raw or moderately processed foods (van Dongen *et al.*, 2012; van Langeveld *et al.*, 2017). This phenomenon has significant implications for food intake and eating behaviour, leading to over-consumption of energy through intakes of highly processed sweet and fatty foods. These clear links between taste perception, dietary intake and metabolic health highlight the need for reformulation of food products to improve diet quality and reduce the passive over-consumption of sugar and fat.

Food reformulation is defined as reformulating existing foods by removing or reducing certain ingredients while maintaining characteristics such as taste, texture and shelf-life (van Raaij *et al.*, 2009). Food reformulation methods include reduction in salt, fat and sugar, replacing trans fats with other oils, replacing saturated with unsaturated fats and designing sugar- and/or fat-free food options (L'Abbé *et al.*, 2009; Réquillart and Soler, 2014). As discussed in the literature review, limited intervention trials show that some aspects of sweet and fat taste perception can be altered by dietary interventions of reduced sweet and fat intakes. We could speculate that by reducing the quantity of sugar and fat gradually over time, consumer's taste perception may get accustomed to healthier food options with lower amounts of sugar and fat. Furthermore, reducing sugar content through food reformulation may be a method which could help to habituate a lower consumption of energy-dense foods through behaviour modification (Yeung *et al.*, 2017). Therefore, public health agencies and policymakers in partnerships with the food industry should work towards reformulation of foods with the aim to produce healthier food products, improve the current food environment and consequently improve population health outcomes (Spiteri and Soler, 2018; European Commission., 2009).

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## 7.4 Final conclusions

The four research studies of this thesis investigated the relationship between sweet taste and fat (creaminess) perception, dietary intake and obesity in well-defined body composition groups using appropriate and valid taste perception and dietary methodology. The findings of this thesis make several important contributions to the field. An important research gap addressed in this thesis was the systematic comparison between four widely used psychophysical measurements of sweet taste perception and actual dietary intake. Past studies have used various psychophysical measurements of taste interchangeably without thoroughly assessing the relationship between these important taste measurements and diet (Holt *et al.*, 2000; Martinez-Cordero *et al.*, 2015; Low *et al.*, 2016). The findings of this thesis showed very clearly that sweet hedonic liking is dependent on the perceived sweetness intensity. Another important finding was the clear link between suprathreshold measurements of sweet taste (i.e., perceived taste intensity and hedonic liking) and dietary intake. Therefore, an important methodological and scientific progression and contribution of this thesis was that suprathreshold taste measurements are most appropriate and valid when assessing relationships with diet, and that these measurements should be used in future studies.

A further important investigation of this thesis was comparing sweet taste and fat (creaminess) perception between ethnic groups with known differences in obesity risk and between well-defined body composition groups. Importantly, the results showed that women from all ethnic groups and body composition groups were able to perceive the same level of sweet and fat taste intensity and have similar sweet and fat hedonic likings. These findings indicated very clearly that at least in the group of healthy women studied in this thesis, sweet and fat taste perception did not directly explain differences in metabolic disease risk or obesity profiles. Therefore, the key link between taste perception and obesity must involve dietary intake.

Accordingly, the relationship between dietary patterns and parameters of metabolic health was assessed. The findings showed that higher intakes of the 'refined and processed' dietary pattern was linked with hyper-insulin secretion and increased adiposity, possibly resulting in hyperleptinaemia and leptin resistance, describing the pathogenesis of obesity. As the PCA method was used to extract dietary patterns, the study participants were not grouped into mutually exclusive groups of distinct dietary patterns. Therefore, differences in metabolic health parameters between dietary patterns could not be assessed to identify the dietary pattern that is more beneficial to health. However, findings from this thesis describe a clear link between higher intakes of an unhealthy dietary pattern (i.e., refined and processed pattern) and adverse

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metabolic health outcomes and highlights the need to further explore avenues to reduce the intake of highly refined and processed foods.

Another significant finding was the ethnic-group based differences in dietary patterns, in particular, that more Māori and Pacific women more strongly followed the 'refined and processed' dietary pattern. As sweet taste and fat (creaminess) perception was not different between the ethnic groups, we could interpret that the differences in dietary patterns are most likely related to other determinants of health, such as socio-economic inequities that are known to have a strong influence on food choice, obesity development and metabolic disease risk (Reremoana *et al.*, 2015; Ministry of Health, 2015c; Southwick *et al.*, 2012). As discussed in Chapter 5, higher intakes of the 'refined and processed' dietary pattern was linked with higher adiposity, hyper-insulineamia and hyper-leptinaemia. We could speculate that higher intakes of dietary patterns such as the 'refined and processed' may contribute to the higher prevalence of obesity and metabolic disease risk observed in Māori and Pacific populations (Ministry of Health, 2017). The findings of the present thesis emphasise the need to reduce health inequities by understanding socio-economic and cultural barriers to healthy eating and healthy lifestyle practices, especially in population groups at greatest risk of developing obesity.

The present thesis showed for the first time a clear relationship between a lower perceived sweet taste intensity and a higher sweet hedonic liking and higher intakes of total energy, carbohydrate and sugar. Furthermore, this thesis identified distinct patterns of sweet and fat hedonic liking and found that sweet likers and creaminess likers have higher intakes of sweet and fatty tasting food groups and favour the 'refined and processed' and 'fats and meat' dietary patterns. Collectively, these findings suggest that sweet and fat taste intensity and hedonic liking are important sensory characteristics linked with dietary intake, especially linked with the type and amount of sweet and fatty foods consumed. Although sweet taste and fat (creaminess) perception was not linked with body composition or metabolic health biomarkers, we could speculate that higher intakes of these dietary patterns may result in adverse metabolic health consequences long-term (Te Morenga *et al.*, 2014; Te Morenga *et al.*, 2013).

One of the main implications of this thesis is the need to modify (lower) learned and/or habitual preferences for sweet and fat tasting foods. One avenue is through lowering the exposure to foods high in sugar and fat during infancy and childhood (e.g., healthy eating during pregnancy and breastfeeding, offering healthy foods during the complementary feeding stage, creating a healthier food environment in schools) (Ventura and Worobey, 2013; Ventura and Mennella, 2011; Ministry of Education, 2014; Rovner *et al.*, 2011; Mennella *et al.*, 2001). These strategies

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may contribute to acclimatising children to healthier food options, so their taste preferences are positively imprinted in early-life and consequently reduce the intake of energy-dense sweet and fatty foods and reduce the predisposition to obesity. Furthermore, the need for improving the healthiness of food environments and implementing policies to change the obesogenic food environment is emphasised by the findings of this thesis (Swinburn *et al.*, 2013). This can be achieved by strategies such as reformulation of food products to improve nutrient profile (Yeung *et al.*, 2017; Spiteri and Soler, 2018), smaller portion sizes to reduce energy intake (Robinson and Kersbergen, 2018), better nutritional information (e.g., healthy star ratings, front-of-pack nutrition label) (Peters *et al.*, 2017; Campos *et al.*, 2011), improved supermarket environment (Luiten *et al.*, 2015; Vandevijvere *et al.*, 2018a) and making healthy food more affordable for all population groups (Eyles *et al.*, 2012; Vandevijvere *et al.*, 2018b). By creating a healthier food environment through reducing obesogenic environmental pressures, we are likely to reduce the passive over-consumption of sugar and fat, and as a result reduce diet-related adverse metabolic health consequences (Vandevijvere and Swinburn, 2014; Roberto *et al.*, 2015).

The main aim of the present thesis was to advance our understanding of the relationship between sweet taste and fat (creaminess) perception and dietary intake, and, how this may influence body composition in women in order to understand factors contributing or leading to obesity. Overall, the findings of this thesis suggest that sweet and fat taste intensity and hedonic liking are clearly linked with dietary intake, but not directly with obesity. Furthermore, new evidence from this research programme describes a pathway to diet-induced metabolic dysregulation that is associated with higher intakes of the 'refined and processed' dietary pattern, particularly in population groups at higher risk of obesity. Although no direct link between sweet taste and fat (creaminess) perception and metabolic health was found, the findings in this thesis suggest that there is an indirect link between higher sweet and fat hedonic liking and adverse metabolic health outcomes, one that is mediated through higher intakes of unhealthy dietary patterns such as the 'refined and processed' pattern. Understanding factors that drive higher intakes of carbohydrates (starch and sugar) and dietary fats is an essential step for learning how to reduce passive over-consumption of energy and consequently obesity development. The overall findings of this thesis highlight the need to break the viscous cycle of a higher sweet and fat hedonic liking and unhealthy food choices, especially in population groups at higher risk of obesity and metabolic disease. As discussed above, promising directions to achieve this include community-based interventions, food policies to reduce obesity, public health initiatives for improving the healthiness of food environments and reduction of socio-economic and health inequities.

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## Appendix 1 Contributions of authors for Chapter 3

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Author	Contribution
Shakeela Jayasinghe School of Sport, Exercise and Nutrition, Massey University, Auckland, New Zealand	Involved in designing all aspects of the study including sensory and dietary measurements, obtained ethics approval, recruited participants, conducted the study, analysed and interpreted data, main author of manuscript.
Professor Bernhard Breier School of Sport, Exercise and Nutrition, Massey University, Auckland, New Zealand	Primary supervisor of PhD, principal investigator and advisor, provided funding for the study, involved in the study design including sensory and dietary measurements, contributed to the interpretation of data and writing of manuscript, revised and approved manuscript.
Associate Professor Rozanne Kruger School of Sport, Exercise and Nutrition, Massey University, Auckland, New Zealand	Co-supervisor of PhD, main advisor of all dietary measurements, involved in the design of dietary questionnaires and food record, and interpretation of dietary data, revised and approved manuscript.
Dr Daniel Walsh Massey University, Auckland, New Zealand	Co-supervisor of PhD, involved in designing the study, assisted with all statistical analysis, revised and approved manuscript.
Stacey Rivers School of Sport, Exercise and Nutrition, Massey University, Auckland, New Zealand	Designed dietary questionnaires, assisted with data collection, revised and approved manuscript.
Maggie Cao School of Sport, Exercise and Nutrition, Massey University, Auckland, New Zealand	Involved in data collection, entered and analysed food records, revised and approved manuscript.
Dr Marilize Richter School of Sport, Exercise and Nutrition, Massey University, Auckland, New Zealand	Assisted with analysing food records, revised and approved manuscript

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## Appendix 2 Statement of contribution to doctoral thesis containing publications

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DRC 16



MASSEY UNIVERSITY  
GRADUATE RESEARCH SCHOOL

### STATEMENT OF CONTRIBUTION TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

**Name of Candidate:** Shakeela Nathalia Jayasinghe

**Name/Title of Principal Supervisor:** Professor Bernhard Breier

**Name of Published Research Output and full reference:**

Jayasinghe, S., Kruger, R., Walsh, D., Cao, G., Rivers, S., Richter, M., & Breier, B. (2017).  
Is Sweet Taste Perception Associated with Sweet Food Liking and Intake? *Nutrients*, 9(7), 750.  
doi:10.3390/nu9070750

**In which Chapter is the Published Work:** Chapter 3

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate:  
and / or
- Describe the contribution that the candidate has made to the Published Work:  
Involved in designing all aspects of the study including sensory and dietary measurements, obtained ethics approval, recruited participants, conducted the study, analysed and interpreted data, main author of manuscript.

Shakeela Nathalia Jayasinghe  
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Candidate's Signature

9/4/2018

Date

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Principal Supervisor's signature

9/4/2018

Date

GRS Version 3– 16 September 2011

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## Appendix 3 Sweet food-food frequency questionnaire

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### Sweet Taste Study FFQ

\* 1. Please enter your subject ID

\* 2. Please enter your name

### Sweet Taste Study FFQ

This questionnaire is designed to obtain information about your usual intake of sweet tasting food in the past month. Please answer by ticking HOW OFTEN you ate a particular food over the LAST MONTH. If you did not consume a type of food over the last month please choose NEVER. Please answer the questionnaire as accurately as possible. Refer to the examples sheet provided if any of the foods are unclear.

### Sweet Taste Study FFQ

Fruit and Vegetables

\* 3. How often do you usually have the following fruits?

	Never	Less than once per month	2-3 times per month	Once a week	2-4 times per week	4-6 times per week	Once a day	Twice a day or more
Apricots	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Apples/Pears	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Bananas	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Berries	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Grapes	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Kiwifruit	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Mango	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Citrus fruit	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Pineapple	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Feijoa	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Stone fruit	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Pears	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Melon (water melon or rockmelon), paw-paw	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Dried Fruit	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Canned fruit in syrup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Canned fruit in juice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

\* 4. How often do you have the following vegetables

	Never	Less than once per month	2-3 times per month	Once a week	2-4 times per week	4-6 times per week	Once a day	Twice a day or more
Beetroot	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Corn	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Pumpkin	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Kumara (yellow or orange)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

**Sweet Taste Study FFQ**

Dairy based foods, Cereals, Spreads and Sweeteners

\* 5. How often do you consume the following dairy based foods?

	Never	Less than once per month	2-3 times per month	Once a week	2-4 times per week	4-6 times per week	Once a day	Twice a day or more
Yoghurt / frozen yoghurt	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Dairy food	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Yoghurt drinks	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Flavoured milk/milkshakes	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

\* 6. How often do you have the following cereals?

	Never	Less than once per month	2-3 times per month	Once a week	2-4 times per week	4-6 times per week	Once a day	Twice a day or more
Muesli	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Natural cereals (All bran, Special K)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Light and fruity cereals (Just Right, Light and Tasty)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Chocolate based cereals (Coco Pops, Milo cereal)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Liquid breakfast (Up and Go)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

\* 7. How often do you have the following spreads or sweeteners?

	Never	Less than once per month	2-3 times per month	Once a week	2-4 times per week	4-6 times per week	Once a day	Twice a day or more
Nutella	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Jam	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Marmalade	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Honey/ Golden syrup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sugar, white	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Alternative sweetener	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

**Sweet Taste Study FFQ**

Cakes, biscuits, sweet foods, desserts

\* 8. How often do you have the following foods?

	Never	Less than once per month	2-3 times per month	Once a week	2-4 times per week	4-6 times per week	Once a day	Twice a day or more
Cake	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Cheesecake	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Loaves	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Pastries/Pinwheels (sweet)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Scone (sweet)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Iced buns/twist	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Tarts	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Plain biscuits	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Chocolate or cream biscuits	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Waffles, pancakes or pikelets	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Muesli bars, breakfast bars or energy bars	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Chocolate	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Hard boiled lollies	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Soft lollies	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

\* 9. How often do you have the following desserts?

	Never	Less than once per month	2-3 times per month	Once a week	2-4 times per week	4-6 times per week	Once a day	Twice a day or more
Jelly	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Icecream	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Ice blocks	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sorbet	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Custard	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Dairy desserts (instant pudding)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

Sweet Taste Study FFQ

Beverages

\* 10. How often do you have the following beverages?

	Never	Less than once per month	2-3 times per month	Once a week	2-4 times per week	4-6 times per week	Once a day	Twice a day or more
Fruit juice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Fruit drink	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Cordial	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Soft drink, regular	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Soft drink, sugar free or diet	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Iced Coffee	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Milk mixer	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Ice tea	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Fruit Smoothie	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Yoghurt drink	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Flavoured water	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Beer,lagar or cider	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Dessert wine	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Spirit with mixer	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Cocktails	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

**Sweet Taste Study FFQ**

\* 11. What is your most favourite food to eat and how often do you have it?

Most favourite food:

How often?

\* 12. What are the three most popular foods you would buy when away from home? Name 3 in order of preference.

1

2

3

\* 13. Do you usually snack during the day?

- Yes  
 No

\* 14. Name your three favourite snacks (e.g. chips, cake, cheese)?

1

2

3

\* 15. If you are having a snack to eat would you prefer something sweet or savoury?

- Sweet  
 Savoury

\* 16. Do you like/enjoy sweet food?

- Yes  
 No

\* 17. Do you believe you have a 'sweet tooth'?

- Yes  
 No

#### Sweet Taste Study FFQ

\* 18. If yes, why do you think you have a sweet tooth?

#### Sweet Taste Study FFQ

\* 19. Do you regularly experience food cravings?

Yes

No

**Sweet Taste Study FFQ**

\* 20. If yes, what type of food do you crave, give two examples

1

2

**Sweet Taste Study FFQ**

\* 21. What is your 3 most favourite sweet foods (not drinks) and how often do you have it?

Food type 1

How often?

Food type 2

How often?

Food type 3

How often?

**Sweet Taste Study FFQ**

\* 22. Do you have sugar in your hot drinks?

Yes

No

**Sweet Taste Study FFQ**

\* 23. If yes, how many teaspoons per cup of hot beverage?

**Sweet Taste Study FFQ**

\* 24. Do you have sugar on your cereal?

Yes

No

**Sweet Taste Study FFQ**

\* 25. If yes, how many teaspoons?

**Sweet Taste Study FFQ**

**Thank you for taking the time to complete this food frequency questionnaire**

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## Appendix 4 Contributions of the EXPLORE study team

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Researcher	Contribution
Shakeela Jayasinghe School of Sport, Exercise and Nutrition, Massey University, Auckland, New Zealand	Developed and led all aspects of sensory analysis, recruited and screened participants, involved in all aspects of data collection, conducted all statistical analysis, interpretation of data and write up of EXPLORE study experimental chapters
Professor Bernhard Breier School of Sport, Exercise and Nutrition, Massey University, Auckland, New Zealand	Primary supervisor of PhD, guided all sensory and metabolic biomarker measurements, involved in interpretation of results and data analysis, revised and approved all EXPLORE study chapters
Associate Professor Rozanne Kruger School of Sport, Exercise and Nutrition, Massey University, Auckland, New Zealand	Co-supervisor of PhD, principal investigator of the EXPLORE study, developed the study design and obtained ethics approval, primary advisor of all dietary analysis, involved in data analysis and interpretation, revised and approved EXPLORE study chapters
Dr Daniel Walsh Massey University, Auckland, New Zealand	Co-supervisor of PhD, involved in statistical analysis and interpretation, revised and approved EXPLORE study chapters
Dr Lily George Massey University, New Zealand	Advised on the cultural aspects and recruitment of Māori women
Dr Riz Firestone Massey University, New Zealand	Advised on the cultural aspects and recruitment of Pacific women
Professor Aaron Russell Deakin University, Melbourne, Australia	Advised and performed all metabolic biomarker analysis
Professor Sarah McNaughton Deakin University, Melbourne, Australia	Advised and guided dietary pattern analysis

# Appendix 5 Food frequency questionnaire

## EXPLORE Food Frequency Questionnaire

1. Please read carefully before you begin:

Please make sure when filling out this questionnaire that you:

- Tell us what YOU usually eat (not someone else in your household!).
- Fill in the form YOURSELF.
- Are correct, but don't spend too much time on each food.
- Answer EVERY question; the asterisk symbol (\*) at the beginning of each question means that you must answer before moving onto the next question.

This will help us to get the most accurate information about your usual food intake.

Please answer by ticking the box which best describes HOW OFTEN you ate or drank a particular food or drink in the LAST MONTH and HOW MUCH you would usually have.

For example:

1. EXAMPLE: How often do you usually have sugar? (Please do not fill out)

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Sugar - 1 tsp	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

If every day you have 2 cups of coffee with 1 tsp sugar, 4 cups of tea with 1 tsp sugar, one bowl of cereal with 1 tsp sugar and sugar on pancakes at dinner, you would choose four or more times per day = '4+ x / day'.

Adjust your portion size and frequency of intake to suit your eating habits.

2. EXAMPLE: How often do you usually eat bread? (Please do not fill out)

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Bread - 1 slice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

If every day you have two slices of toast for breakfast, and you have a sandwich for lunch three times per week, you would choose two - three times per day = '2-3x / day'.

Adjust your portion size and frequency of intake to suit your eating habits.

## EXPLORE Food Frequency Questionnaire

2. EXPLORE Study Food Frequency Questionnaire

\* 1. Please enter your study ID (if you are unsure or don't know please ask the researcher)

### EXPLORE Food Frequency Questionnaire

#### 3. Eating Pattern

\* 1. How would you describe your eating pattern? (Please choose one only)

- Eat a variety of all foods, including animal products
- Eat eggs, dairy products, fish and chicken but avoid other meats
- Eat eggs, dairy products and fish, but avoid chicken and other red meats
- Eat eggs and dairy products, but avoid all meats, chicken and fish
- Eat eggs, but avoid dairy products, all meats and fish
- Eat dairy products, but avoid eggs, all meats and fish
- Eat no animal products
- None of the above

Other (please state)

### EXPLORE Food Frequency Questionnaire

#### 4. Dairy

\* 1. Do you use milk? (e.g. fresh, UHT, powdered)

- Yes
- No

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\* 2. What type(s) of milk do you have most often? (You can choose up to 3 options, but please only choose the ones you usually have)

- Not applicable
- Full cream milk (purple top)
- Standard milk (blue top)
- Skim milk (light blue top)
- Trim milk (green top)
- Super trim milk (light green top)
- Calcium enriched milk (yellow top) e.g. Xtra, Calci-Trim
- Calcium and vitamin enriched milk e.g. Mega, Anlene
- Calcium and protein enriched milk e.g. Sun Latte
- Standard soy milk (blue)
- Light soy milk (light blue)
- Calcium enriched soy milk (purple) e.g. Calci-Forte, Calci-Plus
- Calcium, vitamin and omega 3 enriched soy milk e.g. Essential
- Calcium and high fibre enriched soy milk e.g. Calci-Plus High Fibre
- Rice milk

Other (please state)

\* 3. Choose the one milk you have the most

- Not applicable
- Full cream milk (purple top)
- Standard milk (blue top)
- Skim milk (light blue)
- Trim milk (green top)
- Super trim milk (light green top)
- Calcium enriched milk (yellow top) e.g. Xtra, Calci-Trim
- Calcium and vitamin enriched milk e.g. Mega, Anlene
- Calcium and protein enriched milk e.g. Sun Latte
- Standard soy milk (blue)
- Light soy milk (light blue)
- Calcium enriched soy milk (purple) e.g. Calci-Forte, Calci-Plus
- Calcium, vitamin and omega 3 enriched soy milk e.g. Essential
- Calcium and high fibre enriched soy milk e.g. Calci-Plus High Fibre
- Rice milk

Other (please state)

\* 4. On average, how many servings of milk do you have per day? (Please choose one only)

(A 'serving' = 250 mL or 1 cup/glass)

e.g. 5 cups of coffee/tea using 50 mL of milk + ½ cup of milk on cereal = 1 ½ servings per day

- Not applicable
- Less than 1 serving
- 1-2 servings
- 3-4 servings
- 5 or more servings

\* 5. How often do you usually have milk?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Flavoured milk (milkshake, iced coffee, Primo, Nesquik) - 250 mL / 1 cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Milk as a drink - 250 mL / 1 cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Milk on breakfast cereals or porridge - 125 mL / 1/2 cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Milk added to water-based hot drinks (coffee, tea) - 50 mL / 1/5 cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Milk-based hot drinks (Latte, Milo) - 250 mL / 1 cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

\* 6. How often do you usually eat cheese?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day	4+ x / day
Cheddar (tasty, mild, colby) - 2 heaped Tbsp / matchbox cube	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Edam, Gouda, Swiss - 2 heaped Tbsp / matchbox cube	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Feta, Mozzarella, Camembert - 1 heaped Tbsp / 1 med wedge	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Brie, blue and other specialty cheese - 1 heaped Tbsp / 1 med wedge	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Processed cheese slices - 1 slice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Cream cheese - 2 heaped Tbsp	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Cottage or ricotta cheese - 2 heaped Tbsp	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

\* 7. How often do you usually eat these dairy based foods?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Ice cream - 2 scoops	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Custard or dairy food - 1 pottle / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Yoghurt, plain or flavour - 1 pottle / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Milk puddings (semolina, instant) - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Fermented or evaporated milk (buttermilk) - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

**EXPLORE Food Frequency Questionnaire**

5. Bread

\* 1. Do you eat bread?

- No
- Yes

\* 2. What type(s) of bread, rolls or toast do you eat most often? (You can choose up to 3 options, but please only choose the ones you usually have)

- Not applicable
- White
- White – high fibre
- Wholemeal or wheat meal
- Wholegrain

Other (please state)

\* 3. What type of bread slice do you usually have? (Please choose one only)

- Not applicable
- Sandwich slice
- Toast slice
- Mixture of both sandwich and toast slices

\* 4. On average, how many servings of bread do eat per day? (Please choose one only)

(A 'serving' = 1 slice of bread or 1 small roll)

- Not applicable
- Less than 1 serving
- 1–2 servings
- 3–4 servings
- 5–6 servings
- 7 or more servings

\* 5. How often do you usually eat these bread based foods?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Plain white bread - 1 slice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
High fibre white bread - 1 slice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Wholemeal or wheat meal - 1 slice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Wholegrain bread - 1 slice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Fruit bread or fruit bun - 1 slice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Wrap - 1 medium	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Focaccia, bagel, pita, panini or other speciality breads - 1 medium	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Paraoa Parai (fry bread) - 1 slice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Rewena bread - 1 slice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Doughboys or Maori bread - 1 slice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

\* 6. How often do you usually eat these other bread based foods?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Crumpet or muffin split - 1 crumpet / 1 whole muffin split	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Scone - 1 medium	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Bran muffin or savoury muffin - 1 medium	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Croissant - 1 medium	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Waffle, pancakes or pikelets - 1 medium / 2 small	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Iced buns - 1 medium	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Crackers (cream crackers, cruskits, corn / rice crackers, vitawheat) - 2 medium	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

\* 7. Do you have butter, margarine or spreads on bread or crackers?

- No
- Yes

\* 8. What type(s) do you have most often? (You can choose up to 3 options, but please only choose the ones you usually have)

- Not applicable
- Butter (all varieties)
- Monounsaturated fat margarine e.g. Olive, Rice Bran, Canola Oil Spreads
- Polyunsaturated fat margarine e.g. Sunflower Oil Spreads
- Light monounsaturated fat margarine e.g. Olivio Spread Light
- Light polyunsaturated fat margarine e.g. Flora Spread Light
- Plant sterol enriched margarine e.g. Pro Active, Logical Spreads
- Light plant sterol enriched margarine e.g. Pro Active Spread Light
- Butter and margarine blend e.g. Country Soft, Butter Lea

Other (please state)

\* 9. Choose the one you have the most

- Not applicable
- Butter (all varieties)
- Monounsaturated fat margarine e.g. Olive, Rice Bran, Canola Oil Spreads
- Polyunsaturated fat margarine e.g. Sunflower Oil Spreads
- Light monounsaturated fat margarine e.g. Olivio Spread Light
- Light polyunsaturated fat margarine e.g. Flora Spread Light
- Plant sterol enriched margarine e.g. Pro Active, Logical Spreads
- Light plant sterol enriched margarine e.g. Pro Active Spread Light
- Butter and margarine blend e.g. Country Soft, Butter Lea
- Other (please state)

\* 10. On average, how many servings of butter, margarine or spreads do you have per day? (Please choose one only)

(A 'serving' = 1 level teaspoon or 5 mL)

e.g. 1 sandwich with butter thinly spread on two pieces of bread = 2 servings

- Not applicable
- Less than 1 serving
- 1–2 servings
- 3–4 servings
- 5–6 servings
- 7 or more servings

## EXPLORE Food Frequency Questionnaire

### 6. Breakfast Cereals and Porridge

\* 1. Do you usually eat breakfast cereal and/or porridge?

- No
- Yes

\* 2. What breakfast cereal(s) do you eat most often? (You can choose up to 3 options, but please only choose the ones you usually have)

- Not applicable
- Weetbix
- Refined cereals e.g. Cornflakes or Rice Bubbles
- Bran based cereals including fruity varieties e.g. Special K, Muesli, All Bran
- Sweetened e.g. Nutrigrain, Cocoa Pops
- Porridge

Other (please state)

\* 3. On average, how many servings of breakfast cereal or porridge do you have per week? (Please choose one only)

(A 'serving' = ½ cup porridge, muesli, cornflakes or 2 weetbix)

e.g. ½ cup of porridge 3 times per week + 2 weetbix 4 times a week = 7 servings per week

- Not applicable
- Less than 4 servings
- 4–6 servings
- 7–9 servings
- 10–12 servings
- 13–15 servings
- 16 or more servings

\* 4. How often do you usually eat porridge or these cereal foods?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Porridge, rolled oats, oat bran, oat meal - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Muesli (all varieties) - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Weetbix (all varieties) - 2 weetbix	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Cornflakes or rice bubbles - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Bran cereals (All Bran, Bran Flakes) - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Bran based cereals (Sultana Bran, Sultana Bran Extra) - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Light and fruity cereals (Special K, Light and Tasty) - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Chocolate based cereals (Milo cereal, Coco Pops) - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sweetened cereals (Nutrigrain, Fruit Loops, Honey Puffs, Frosties) - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Breakfast drinks (Up and Go) - Small carton / 250 mL	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

## EXPLORE Food Frequency Questionnaire

### 7. Starchy Foods

\* 1. Do you eat any type of starchy foods such as rice, pasta, noodles and couscous?

- No
- Yes

\* 2. On average, how many servings of starchy foods such as rice, pasta, noodles and couscous do you eat per week? (Please choose one only)

(A 'serving' = 1 cup cooked rice / pasta)

e.g. 1 cup of rice + ½ cup of pasta included in a lasagne pasta dish + 1 cup of spaghetti = 2.5 servings

- Not applicable
- Less than 4 servings
- 4–6 servings
- 7–9 servings
- 10–12 servings
- 13–15 servings
- 16 or more servings

\* 3. How often do you usually eat these starchy foods?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Rice, white - 1 cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Rice, brown or wild - 1 cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Pasta, white or wholegrain (spaghetti, vermicelli) - 1 cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Canned spaghetti (Watties) - 1 cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Instant noodles (2 minute noodles) - 1 packet	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Egg and rice noodles (hokkien noodles, udon) - 1 cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Other grain (quinoa, couscous, bulgar wheat) - 1 cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

## EXPLORE Food Frequency Questionnaire

### 8. Meat

\* 1. Do you eat beef, mutton, hogget, lamb, or pork

- No
- Yes

\* 2. Do you trim any excess fat (fat you can see) off these meats? (Please choose one only)

- Not applicable
- Always
- Often
- Occasionally
- Never cut the fat off meat

\* 3. On average, how many servings of meat e.g. beef, mutton, hogget, lamb or pork do you eat per week? (Please choose one only)

(A 'serving' = palm size or ½ a cup of meat without bone)  
 e.g. ½ cup of savoury mince + 2 small lamb chops = 2 servings

- Not applicable
- Less than 1 serving
- 1-3 servings
- 4-6 servings
- 7 or more servings

\* 4. How often do you usually eat meat?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Beef mince dishes (rissoles, meatloaf, hamburger pattie) - 1 slice / patty / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Beef or veal mixed dishes (casserole, stir-fry) - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Beef or veal (roast, chop, steak, schnitzel, corned beef) - palm size / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Lamb, hogget or mutton mixed dishes (stews, casserole, stir-fry) - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Lamb, hogget or mutton (roast, chops, steak) - palm size / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Pork (roast, chop, steak) - palm size / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Canned corned beef - 1 medium slice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

\* 5. How often do you usually eat these other meats?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Sausage, frankfurter or saveloy - 1 sausage / frankfurter/ 2 saveloys	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Bacon - 2 rashers	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Ham - 1 medium slice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Luncheon meats or brawn - 1 slice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Salami or chorizo - 1 slice / cube	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Offal (liver, kidneys, pate) - palm size / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Venison/game - palm size / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

## EXPLORE Food Frequency Questionnaire

### 9. Poultry

\* 1. Do you eat poultry e.g. chicken, turkey or duck?

- No  
 Yes

\* 2. Do you remove the skin from chicken? (Please choose one only)

- Not applicable  
 Always  
 Often  
 Occasionally  
 Never remove the skin from chicken

\* 3. On average, how many servings of chicken do you eat per week? (Please choose one only)

(A 'serving' = palm size of chicken or ½ cup)

e.g. 1 chicken breast + 2 chicken drumsticks + 1 chicken thigh = 4 servings per week

- Not applicable  
 Less than 1 serving  
 1-3 servings  
 4-6 servings  
 7 or more servings

\* 4. How often do you usually eat poultry?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Chicken legs or wings - palm size / ½ cup / 1 unit (wing, drumstick)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Chicken breast - palm size / ½ cup / ½ breast	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Chicken mixed dishes (casserole, stir-fry) - palm size / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Crumbed chicken (nuggets, patties, schnitzel) - 1 medium / 4 nuggets	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Turkey or quail - palm size / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Mutton bird or duck - palm size / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

## EXPLORE Food Frequency Questionnaire

### 10. Fish and Seafood

\* 1. Do you eat any type of fish or seafood?

- No  
 Yes

\* 2. On average, how many servings of fish and seafood (all types; fresh, frozen, tinned) do you eat per week? (Please choose one only)

(A 'serving' = 80 - 120g or palm size or small tin (85g))

e.g. 1 fish fillet and 1 small tin of tuna = 2 servings per week.

- Not applicable  
 Less than 1 serving  
 1-3 servings  
 4-6 servings  
 7 or more servings

\* 3. How do you normally cook / eat fish? (You can choose up to 3 options, but please only choose the ones you usually have)

- Not applicable
- Raw / I don't cook it
- Oven baked / Grilled
- Deep fried
- Shallow fry
- Micro waved
- Steamed
- Poached
- Smoked

\* 4. How often do you usually eat seafood?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Canned Salmon - 1 small can (85-95g)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Canned Tuna - 1 small can (85-95g)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Canned Mackerel, sardines, anchovies, herring - 1 small can (85-95g)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Frozen crumbed fish (patties, filets, cakes, fingers, nuggets) - 1 medium / 4 nuggets	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Snapper, Tarakihi, Hoki, Cod, Flounder - palm size / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Gurnard, Kahawai or Trevally - palm size / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Lemon fish or Shark - palm size / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Tuna - palm size / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Salmon, trout or eel - palm size / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

\* 5. How often do you usually eat seafood?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Shrimp, prawn, lobster or crayfish - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Crab or surumi - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Scallops, mussels, oysters, paua or clams - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Pipi or cockle - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Kina - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Whitebait - ¼ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Roe - ¼ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Squid, octopus, calamari, cuttlefish - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

## EXPLORE Food Frequency Questionnaire

### 11. Fats and Oils

\* 1. Do you cook meat, chicken, fish, eggs and/or vegetables with fat or oil?

- No  
 Yes

\* 2. What type(s) do you use most often? (You can choose up to 3 options, but please only choose the ones you usually have)

- Not applicable  
 Butter (all varieties)  
 Margarines (all varieties)  
 Cooking oils (all varieties)  
 Lard, Dripping, Coconut oil, Ghee (clarified butter)  
 Cooking spray

Other (please state)

\* 3. Chose the one you use the most

- Not applicable
- Butter (all varieties)
- Margarines (all varieties)
- Cooking oils (all varieties)
- Lard, Dripping, Coconut oil, Ghee (clarified butter)
- Cooking spray
- Other (please state)

\* 4. When you use fat or oil to cook, how many servings of fat or oil do you use per dish? (Please choose one only)

(A 'serving' = 1 level teaspoon or 5 mL)

- Not applicable
- Less than 1 serving
- 1 serving
- 2 servings
- 3 servings
- 4 servings
- 5 or more servings

\* 5. On average, how many servings of fat or oil do you use to cook per week? (Please choose one only)

- Not applicable
- Less than 1 serving
- 1-3 servings
- 4-7 servings
- 8-10 servings
- 11-14 servings
- 15 or more servings

## EXPLORE Food Frequency Questionnaire

### 12. Eggs

\* 1. Do you eat eggs?

- No  
 Yes

\* 2. On average, not counting eggs used in baking / cooking, how many eggs do you usually eat per week? (Please choose one only)

- Not applicable  
 Less than 1 egg  
 1 egg  
 2 eggs  
 3 eggs  
 4 eggs  
 5 or more eggs

\* 3. How often do you usually eat eggs?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Whole eggs (hard-boiled, poached, fried, mashed, omelette, scrambled) - 1 egg	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Mixed egg dish (quiche, frittata, other baked egg) - 1 slice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

## EXPLORE Food Frequency Questionnaire

### 13. Legumes

\* 1. Do you eat legumes e.g. chickpeas/dried peas, soybeans, dried/canned beans, baked beans, lentils or Dahl?

- No  
 Yes

\* 2. On average, how many servings of legumes (fresh, frozen, canned, dried) do you eat per week?

(Please choose one only)

(A 'serving' = ½ cup or 125g of cooked legumes)

- Not applicable
- Less than 1 serving
- 1 serving
- 2 servings
- 3 servings
- 4-5 servings
- 6-7 servings
- 8 or more servings

\* 3. How often do you usually eat these legumes?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Soybeans - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Tofu - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Dahl - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Canned or dried legumes, beans (baked beans, chickpeas, lentils, peas, beans) - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Hummus - 2 Tbsp	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

## EXPLORE Food Frequency Questionnaire

### 14. Vegetables

\* 1. Do you eat vegetables?

- No
- Yes

\* 2. On average, how many servings of vegetables (fresh, frozen, canned) do you eat per day? Do NOT include vegetable juices. (Please choose one only)

(A 'serving' = 1 medium potato / kumara or ½ cup cooked vegetables or 1/2 cup of lettuce)  
e.g. 2 medium potatoes + ½ cup of peas = 3 servings

- Not applicable
- Less than 1 serving
- 1 serving
- 2 servings
- 3 servings
- 4 or more servings

\* 3. How often do you usually eat these vegetables?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Potato (boiled, mashed, baked, roasted) - 1 medium / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Pumpkin (boiled, mashed, baked, roasted) - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Kumara (boiled, mashed, baked, roasted) - 1 medium / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Mixed frozen vegetables - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Green beans - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Silver beet, spinach - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Carrots - 1 medium / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sweet corn - 1 medium cob / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Mushrooms - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Tomatoes - 1 medium / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Beetroot - 1 medium / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Taro, cassava or breadfruit - 1 medium / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

\* 4. How often do you usually eat these vegetables?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Green bananas (plantain) - 1 medium / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sprouts (alfalfa, mung) - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Pacific Island yams - 1 medium / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Turnips, swedes, parsnip or yams - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Onions, celery or leeks - ¼ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Cauliflower, broccoli or broccoflower - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Brussel sprouts, cabbage, red cabbage or kale - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Courgette/zucchini, marrow, eggplant, squash, kamo kamo, asparagus, cucumber - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Capsicum (peppers) - ½ medium / ¼ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Avocado - ¼ avocado	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Lettuce greens (mesculin, cos, iceberg) - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Other green leafy vegetables (whitloof, watercress, taro leaves, puha) - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

## EXPLORE Food Frequency Questionnaire

### 15. Fruit

\* 1. Do you eat fruit?

- No  
 Yes

\* 2. On average, how many servings of fruit (fresh, frozen, canned or stewed) do you eat per day? Do NOT include fruit juice. (Please choose one only)

(A 'serving' = 1 medium or 2 small pieces of fruit or 1/2 cup of chopped fruit)

e.g. 1 apple + 2 small apricots = 2 servings)

- Not applicable  
 Less than one serving  
 1 serving  
 2 servings  
 3 or more servings

\* 3. How often do you usually eat these fruits?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Apple - 1 medium / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Pear - 1 medium / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Banana - 1 medium / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Orange, mandarin, tangelo, grapefruit - 1 medium / 2 small	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Peach, nectarine, plum or apricot - 1 medium / ½ cup / 2 small	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Mango, paw-paw or persimmons / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Pineapple - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Grapes - ½ cup / 8-10 grapes	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Strawberries, other berries, cherries - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Melon (watermelon, rockmelon) - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Kiwifruit - 1 medium / 2 small	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Feijoas - 1 medium / 2 small	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Tamarillos - 1 medium / ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sultanas, raisins or currants - 1 small box	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Other dried fruit (apricots, prunes, dates) - 4 pieces	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

## EXPLORE Food Frequency Questionnaire

### 16. Drinks

\* 1. On average, how many drinks do you have per day? (Please choose one only)  
(A 'serving' = 250 mL or one cup/glass)

- Less than 1 serving
- 1-3 servings
- 4-5 servings
- 6-8 servings
- 9-10 servings
- 11 or more servings

\* 2. How often do you usually have these drinks?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Instant soup (Cup of soup) - 250 mL / 1 cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Fruit juice (Just Juice, Fresh-up, Charlie's, Rio Gold) - 250 mL / 1 cup/glass	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Fruit drink (Choice, Rio Spice) - 250 mL / 1 cup/glass	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Vegetable juice (tomato juice, V8 juice) - 250 mL / 1 cup/glass	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Iced Tea (Lipton ice tea) - 250 mL / 1 cup/glass	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Cordial or Powdered drinks (Thriftee, Raro, Vita-fresh) - 250 mL / 1 cup/glass	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Low-calorie cordial - 250 mL / 1 cup/glass	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Energy drinks small-medium can (V, Red Bull) - 250-350 mL	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Energy drinks large can (Monster, Mother, Demon, large V) - 450-550 mL	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sugar-free Energy drinks (sugar-free V, Monster, Red Bull) - 1 small can	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Diet soft/fizzy/carbonated drink (diet sprite) - 250 mL / 1 cup/glass	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Soft/fizzy/carbonated drinks (Coke, Sprite) - 250 mL / 1 cup/glass	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sport's drinks (Gatorade, Powerade) - 1 bottle	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Flavoured water (Mizone, H2Go flavoured) - 1 bottle	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Water (unflavoured mineral water, soda water, tap water) - 250 mL / 1 cup/glass	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

\* 3. How often do you usually have these drinks?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Coffee instant or brewed with or without milk (Nescafe, espresso) - 1 cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Specialty coffees (flat white, cappuccino, lattes) - 1 small cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Coffee decaffeinated or substitute (Inka) - 1 cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Hot chocolate drinks (drinking chocolate, hot chocolate, Koko) - 1 cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Milo - 1 tsp	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Tea (English breakfast tea, Earl Grey) - 1 cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Herbal tea or Green tea - 1 cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Soy drinks - 1 cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

\* 4. How often do you usually have these alcoholic drinks?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Beer – low alcohol - 1 can or bottle	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Beer – ordinary - 1 can or bottle	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Red wine - 1 small glass	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
White wine, champagne, sparkling wine - 1 small glass	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Wine cooler - 1 small glass / bottle	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sparkling grape juice - 1 glass / cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sherry or port - 100 mL	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Spirits, liqueurs - 1 shot or 30 mL	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
RTD (KGB, Vodka Cruiser, Woodstock bourbon) - 1 bottle / can	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Cider - 1 glass / cup / bottle	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Kava - 1 glass / cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

## EXPLORE Food Frequency Questionnaire

### 17. Dressings and Sauces

\* 1. How often do you usually have these dressings or sauces?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Butter (all varieties) - 1 tsp	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Margarine (all varieties) - 1 tsp	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Oil (all varieties) - 1 tsp	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Cream or sour cream - 1 Tbsp	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Mayonnaise or creamy dressings (aioli, tartar sauce) - 1 Tbsp	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Low fat/calorie dressing (reduced fat mayonnaise) - 1 Tbsp	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Salad dressing (french, italian) - 1 Tbsp	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sauces (tomato, BBQ, sweet chilli, mint) - 1 Tbsp	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Mustard - 1 Tbsp	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Soy sauce - 1 Tbsp	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Chutney or relish - 1 Tbsp	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Gravy homemade - ¼ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Instant Gravy (e.g. Maggi) - ¼ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
White sauce/cheese sauce - ¼ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

### EXPLORE Food Frequency Questionnaire

#### 18. Miscellaneous - Cakes, Biscuits and Puddings

\* 1. How often do you usually eat these baked products?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Cakes, loaves, sweet muffins - 1 slice / 1 muffin	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sweet pies or pastries, tarts, doughnuts - 1 medium	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Other puddings or desserts - not including milk-based puddings (sticky date pudding, pavlova) - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Plain biscuits, cookies (Round wine, Ginger nut) - 2 biscuits	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Fancy biscuits (chocolate, cream) - 2 biscuits	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

### EXPLORE Food Frequency Questionnaire

#### 19. Miscellaneous

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\* 1. How often do you usually eat these other foods?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Jelly - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Ice blocks - 1 ice block	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Lollies - 2 lollies	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Chocolate - including chocolate bars (Moro bars) - 1 small bar	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sugar added to food and drinks - 1 level tsp	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Jam, honey, marmalade or syrup - 1 level tsp	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Vegemite or marmite - 1 level tsp	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Peanut butter or other nut spreads - 1 level Tbsp	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Brazil nuts or walnuts - 2	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Peanuts - 10	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Other nuts (almonds, cashew, pistachio, macadamia) - 10	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Seeds (pumpkin, sunflower)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Muesli bars - 1 bar	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Coconut cream - ¼ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Coconut milk - ¼ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Lite coconut milk - ¼ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Potato crisps, corn chips, Twisties - ½ cup / handful	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

\* 2. Do you use salt in cooking?

- Never
- Rarely
- Sometimes
- Usually
- Always

\* 3. Do you use salt at the table?

- Never
- Rarely
- Sometimes
- Usually
- Always

## EXPLORE Food Frequency Questionnaire

### 20. Miscellaneous - Takeaways

\* 1. On average, how often do you eat takeaways per week? (Please choose one only)

- Never  
 Less than 1 times  
 1-2 times  
 3-4 times  
 4-6 times  
 More than 7 times

\* 2. How often do you usually eat these takeaway foods?

	Never	<1x / month	1-3x / month	1x / week	2-3x / week	4-6x / week	Once / day	2-3x / day	4+ x / day
Meat pie, sausage roll, other savouries - 1 pie / 2 small sausage rolls or savouries	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Hot potato chips, kumara chips, french fries, wedges - ½ cup	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Chinese - 1 serve	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Indian - 1 serve	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Thai - 1 serve	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Pizza - 1 medium slice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Burgers - 1 medium burger	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Battered fish - 1 piece	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Fried chicken (KFC, Country fried chicken) - 1 medium piece	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Bread based (Kebab, sandwiches, wraps, Pita Pit, Subway) - 1 medium	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

## EXPLORE Food Frequency Questionnaire

### 21. Other

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\* 1. Are there any other foods or drinks that you can think of that you have on a regular basis that was not covered by this questionnaire?

- No  
 Yes

### EXPLORE Food Frequency Questionnaire

#### 22. Other

1. Please list these foods and drinks including; the serving size, and how many times per week you eat or drink these items (e.g. Pizza, 4 slices, one time per week)

## Appendix 6 Food groups obtained from the food frequency questionnaire

Food group	Food items
Full-fat milk	Full-fat milk (purple and dark blue top)
Low-fat milk	Lite and trim milk
Soy milk	Soy milk, almond milk (all non-dairy milk)
Sweetened milk products	Flavoured milk, fermented or evaporated milk, breakfast drinks, hot chocolate drinks, milo
Yoghurt	Yoghurt (plain, flavoured, Greek)
High-fat cheese	Cheddar, processed cheese, cream cheese, blue vein
Low-fat cheese	Edam, feta, mozzarella, camembert, cottage cheese
Apple, banana, orange	Apple, banana, orange
Other fruit	All other fruit (fresh, canned, frozen, dried)
Tomatoes	Tomatoes
Dark-yellow vegetables	Pumpkin, carrot
Green vegetables	Green beans, silver beet, spinach, sprouts, cauliflower, brussel sprouts, cabbage, courgette, lettuce, other green leafy vegetables
Other non-starchy vegetables	Frozen vegetables, mushrooms, beetroot, onions, capsicum
Potatoes	Potato (boiled, mashed, baked, roasted)
Starchy vegetables	Kumara, sweet corn, taro, cassava, breadfruit, green bananas, turnips, swedes, parsnip, yams
White breads	Plain white bread, fruit bread, wraps, focaccia, bagel, pita, paraoa parai, rewena bread, doughboys
Discretionary breads	Crumpet, scone, savoury muffin, croissant, waffles, pancakes, iced buns
Crackers	Crackers (cream crackers, cruskits, rice crackers, vitawheat)
Whole grain breads	High fibre white bread, wholemeal bread, wholegrain bread
Refined grains	White rice, pasta, spaghetti, vermicelli, canned spaghetti, noodles (instant, egg, rice)
Wholegrains	Brown rice, quinoa, couscous, bulgur wheat
Oats	Porridge, rolled oats, oat bran, oat meal
Sweetened cereals	Sultana Bran, light and fruity cereal, chocolate based cereals, nutrigrain, fruit loops
Sweetened cereals	Sultana Bran, light and fruity cereal, chocolate based cereals, nutrigrain, fruit loops

All food groups were derived from the 220-item food frequency questionnaire.

Food group	Food items
Red meats	Beef (mince, casserole, stir-fry, roast, chop, steak, schnitzel), lamb, hogget or mutton mixed dishes (stews, casserole, stir-fry, roast, chops, steak), offal, venison
White meats	Pork (roast, chop, steak), Chicken (legs, wing, drumstick, breast, casserole, stir-fry), turkey, mutton bird, duck, veal
Processed meats	Sausages, frankfurters, saveloys, bacon, ham, luncheon meat, salami, chorizo, corned beef
Fish and seafood	Canned Salmon, canned tuna, canned mackerel, snapper, tarakihi, hoki, cod, gurnard, kahawai, lemon fish /shark, tuna, salmon, shrimp, crab, scallops, pipi, Kina, whitebait, roe
Egg and egg dishes	Whole eggs (hard-boiled, poached, fried, mashed, omelette, scrambled), mixed egg dish (quiche, frittata)
Legumes	Dahl, canned or dried legumes, beans (baked beans, chickpeas, lentils, peas, beans), hummus
Soy products	Soybeans, tofu
Peanut butter and peanuts	Peanut butter and peanuts
Nuts and seeds	Brazil nuts, walnuts, other nuts (almonds, cashew, pistachio, macadamia), seeds (pumpkin, sunflower)
Fats	Butter, lard, drippings
Coconut fats	Coconut cream and milk
Oil and oil based dressings	Avocado, salad dressing (Italian, French), oil (canola, olive oil)
Margarine	Margarine
Creamy dressings	Sour cream, mayonnaise, creamy dressings (aioli, tartar sauce), low-fat/calorie dressing (reduced fat mayonnaise), white sauce, cheese sauce
Sauces	Instant soup, sauces (tomato, BBQ, sweet chilli, mint), mustard, soy sauce, chutney, gravy (homemade, instant Gravy)
Sweet spreads	Jam, honey, marmalade, syrup
Savoury spreads	Vegemite or marmite
Cake and biscuits	Cakes, loaves, sweet muffins, sweet pies, pastries, tarts, doughnuts, plain biscuits, cookies (round wine, ginger nut), fancy biscuits (chocolate, cream)
Puddings and other deserts	Ice cream, custard, dairy food, milk puddings (semolina, instant), other puddings or desserts (sticky date pudding, pavlova), jelly, ice blocks
Sweet snack foods	Lollies, chocolate, muesli bars
Savoury snack foods	Potato crisps, corn chips, twisties
Crumbed and deep-fried food	Crumbed chicken (nuggets, patties, schnitzel), frozen crumbed fish (patties, fillets, cakes, fingers, nuggets), hot potato chips, kumara chips, French fries, wedges, battered fish, fried chicken (KFC, country fried chicken)
Fast-food	Meat pie, sausage roll, Chinese, Indian, Thai, pizza, burgers, bread based (kebab, sandwiches, wraps, pita pit, subway)

All food groups were derived from the 220-item food frequency questionnaire.

<b>Food group</b>	<b>Food items</b>
Fruit and vegetable juice	Fruit juice, vegetable juice
Fruit drinks, soft drinks and other beverages	Fruit drink, soft drinks, iced tea, cordial, energy drinks, sports drinks, flavoured water, sparkling grape juice
Diet drinks	Low calorie cordial, sugar-free energy drinks, diet soft drinks
Tea	Tea and herbal tea
Coffee	Coffee instant, specialty coffees (flat white, cappuccino, lattes), decaffeinated coffee
Beer	Beer (standard and low alcohol)
Wine	Red and white wine
Water	Water (unflavoured mineral water, soda water, tap water)
Spirits and other alcoholic beverages	Sherry, spirits, liqueurs, ready-to-drink alcohol (KGB, vodka cruiser), cider, kava
Sugar added to food and drink	Sugar added to food and drink

All food groups were derived from the 220-item food frequency questionnaire.