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Drivers of obesity: Associations of physical activity, sedentary behaviour and diet on metabolic health and the gut microbiota

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Abstract

Background: Regular physical activity (PA) and limited time spent sedentary are important for almost all aspects of health, including prevention and treatment of obesity.

Aim: To describe the PA and sedentary behaviour (SB) of healthy, lean and obese, Pacific and NZE women, aged 18-45 years; and to explore the associations of PA and SB with diet, BF%, biomarkers of metabolic health, and gut microbiota composition.

Methods: Pacific (*n* = 142) or NZE (*n* = 162) women aged 18–45 years with a self- reported body mass index of either 18.5–25.0 kg/m^2 or ≥30.0 kg/m^2 were recruited. Whole body dual-energy X-ray absorptiometry was used to subsequently stratify participants as either low (<35%) or high (≥35%) BF%. Eight-day accelerometery assessed PA and SB levels. Meeting the PA guidelines was defined as accumulation of ≥ 30 minutes of moderate or greater intensity activity on ≥ 5 days per week OR 150 minutes of moderate to vigorous PA (MVPA) per week. Dietary intake was assessed using a 5-day food record. Fasting blood was analysed for biomarkers of metabolic health, and whole body dual-energy X-ray absorptiometry was used to estimate body composition. Bulk DNA was extracted from faecal samples and the metagenomic sequences associated with the microbiota were analysed using MetaPhlAN and QIIME2 software. Adjusted multivariate regression models were conducted to explore the associations between PA, SB and diet, body composition and biomarkers of metabolic health, and between PA, SB and gut microbiota composition.

Results: Less than half Pacific women were meeting the PA guidelines (high-BF%; 39% and low-BF%; 47%) versus 81% of low-BF% and 65% of high-BF% NZE women. Low-BF% Pacific women were more sedentary than all other women ($p<0.05$): Pacific low- 10.4 and high-BF% 9.93 and NZE low- 9.69 and high-BF% 9.96 hours/day.

Every additional 10-minutes spent in MVPA was associated with 0.9% lower total and trunk fat and 0.7% lower gynoid fat in all women ($p<0.05$). Among Pacific women; every 100 cpm increase in total PA was associated with 6% lower fasting plasma insulin. Every 10-minute increase in MVPA was associated with 8% lower fasting plasma insulin in both ethnic groups ($p<0.05$). Among NZE women, every one-hour increase in sedentary time was associated with 0.8% higher gynoid fat ($p<0.05$), and longer weighted median sedentary bout length was associated with higher BF% (gynoid fat 0.3%, total body 0.4%, trunk 0.4%, android 0.4% and visceral fat 0.4% ($p<0.05$)) and 14% higher C-reactive protein (CRP) ($p<0.05$). No associations between SB and body composition or metabolic markers were found among Pacific women.

There was no significant difference in average total energy intake between Pacific and NZE women or BF% groups. No women were consuming more than the carbohydrate AMDR (>65% total energy). Pacific women's mean daily starch intake was significantly higher than NZE women (g/day, and % total energy intake). Only the NZE low-BF% groups mean fibre intake was above the recommended daily intake of ≥25g/day. All the women that were in the lowest quartile of fibre intake, and particularly the NZE women, had a lower odds of meeting the PA guidelines (OR 0.72 (p=0.008) and OR 0.66 (p=0.021) respectively) compared to women in the top three quartiles of fibre intake. All the women that were in the lowest quartile of polyunsaturated fat intake, especially Pacific women had a lower odds of meeting the PA guidelines compared women in the top three quartiles (0.76, p=0.027 and OR 0.67, p=0.030 respectively).

Among NZE women, every one SD increase in total PA (197 cpm/day) was associated with 36.3% higher relative abundance of *Erysipelotrichaceae* (p=0.031) and 37.9% lower relative abundance of *Verrucomicrobiaceae* (p=0.029). Every one SD increase in SB (1.45 hours/day) was associated with a 28% lower relative abundance of *Erysipelotrichaceae* (p=0.030). Every one SD increase in NZE women's total PA was associated with 23.1% higher *Firmicutes:Bacteroidetes* ratio (p=0.031), whereas among Pacific women, every 1 SD increase in MVPA was associated with 22.8% lower (p=0.034) *Firmicutes:Bacteroidetes* ratio.

Conclusion: Increased time spent in PA of all intensities and breaking-up prolonged SB was associated with healthier body composition and lower metabolic disease risk in Pacific and NZE women. Compared to NZE, the impact of increased total PA on fasting insulin may be greater in Pacific women and inflammation may be a pathway through which SB impacts cardiovascular risk, especially for NZE women. Although higher total PA and lower SB was associated with some aspects of the gut microbiota composition, more needs to be known about the mechanisms driving associations between PA SB and the gut microbiota to enable these findings to be interpreted.

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Lastly, thank you to everyone else who contributed to my PhD journey, what a journey it has been.

Abbreviations

5DFR = Five day food record

AGE = Advanced glycosylated end-products

AMDR = Acceptable macronutrient distribution range

BF% = Body fat percent

BMI = Body mass index

CHO = Total carbohydrate

cpm = Counts per minute

CRP = C-reactive protein

CVD = Cardiovascular disease

DLW = doubly-labelled water

DXA = Dual-energy x-ray absorptiometry

EDTA = Ethylene diamine tetraacetic acid

EE = Energy expenditure

FFQ = Food frequency questionnaire

HbA1c = Blood glycated haemoglobin

HDL – High density lipoprotein

HOMA-IR = Homeostatic Model Assessment for Insulin Resistance

LDL = Low density lipoprotein

MET = Metabolic equivalent

MVPA = Moderate-to-vigorous intensity physical activity

NCD = Non-communicable disease

NZDep2013 = New Zealand's socioeconomic deprivation index

OECD = The Organisation for Economic Cooperation and Development

OR = Odds ratio

OTU = Operational taxonomic unit

PA = Physical activity

PROMISE = The PRedictors linking Obesity and gut MIcrobiomE study

RPE = Rating of Perceived Exertion

SB = Sedentary behaviour

SBRN = Sedentary Behaviour Research Network

SCFA = Short chain fatty acid

SES = Socioeconomic status

ST = Sedentary time

STAMP = Statistical analysis of metagenomic profiles

T2D = Type 2 diabetes

TEE = Total energy expenditure

USA = United States of America

WC = Waist circumference

WHO = World Health Organisation

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Chapter 1: General introduction – scope and rationale for thesis

Healthy habits, including diet and physical activity (PA) are the foundations for overall health and wellbeing. Over the past half century, with increasing urbanisation and technological advances a dramatic change in the environment has occurred, resulting in increased availability of cheap energy-dense food as well as reduced energy expenditure needed for everyday life (Swinburn et al., 2011). Parallel to this, the prevalence of obesity has increased at an alarming rate (World Health Organisation, 2021). In response to the rapid rise in obesity prevalence at the end of the 20th century, the World Health Organisation declared obesity to be an epidemic. Unfortunately, despite major public health efforts to curb this trend; obesity prevalence continues to rise (World Health Organisation, 2021).

Trends in obesity show a significant rise in prevalence amongst women, with major increased BMI seen between the ages of 20-40 years (The GBD 2015 Obesity Collaborators, 2017). The long-term health impact is concerning because increased adiposity in women of childbearing age is associated with acute and chronic adverse health outcomes (e.g. infertility), including the perpetuation of increased obesity risk for the next generation (Eriksson et al., 2014). These health outcomes are all associated with decreased metabolic health including poor blood lipid profiles, increased blood pressure, inflammation, insulin resistance or type-2 diabetes.

New Zealand (NZ) is boasting some of the highest obesity rates in the world (OECD, 2017). In 1977, 10% of adults living in NZ were considered obese, this figure was doubled just over 20 years later (2003), rising to 21% (Ministry of Health, 2004) and between 2020 and 2021 a further increase to over one third (34.3%) of NZ adults (≥15years) classified as obese (body mass index (BMI ≥30 kg/m^2, or International Obesity Task Force equivalent for 15-17 years; BMI > 2 standard deviations above the WHO growth standard median) (Ministry of Health New Zealand, 2021). Further, when stratifying NZ obesity prevalence by ethnicity, the adult prevalence rates vary considerably and are associated with substantial health inequalities. NZ Pacific Peoples and the NZ Māori population are estimated to have the highest obesity rates at 71.3% and 50.8% respectively, Asian the lowest (18.5%), whereas the NZ European/Other adult population is estimated to have 31.9% obesity prevalence (BMI ≥30 kg/m2 or International Obesity Task Force equivalent for 15-17 years) (Ministry of Health New Zealand, 2021). The reason for this significant difference is unclear but it is hypothesised that it may be linked to genetic variance and or social/environmental differences between the populations (Swinburn et al., 2011). Importantly, there is a strong relationship between obesity and low

socioeconomic status (SES) in NZ (Ministry of Health New Zealand, 2021), and Pacific and Māori people disproportionately live in low SES areas in this country (Ministry of Health New Zealand, 2021). One approach for estimating socioeconomic status is by using the degree of deprivation of an individual's area of residence. Area level deprivation is an aggregated index that measures multiple socio-economic indicators such as income, employment, housing, education, access to transport etc., in NZ this composite measure is called the NZ Deprivation Index (NZDep2013) (Atkinson J, 2014). In the context of this study we will be using NZDep2013 as a proxy for individual SES.

Although theoretically the cause of fat mass gain is simple; a past energy intake in excess of energy expenditure, the drivers of obesity are more complex. Key drivers include societal cues encouraging overconsumption of highly palatable, energy dense and nutrient poor foods and low levels of PA (Swinburn et al., 2011). The global rise in obesity prevalence has increased at a much faster rate than humans can adapt, leading to the notion that this is driven by the contemporary environment having a negative impact on diet and PA behaviours (Swinburn et al., 2011). Therefore, in order to slow the increase in obesity and turn around the epidemic, a larger evidence base supporting the impact of people's choices of both PA and food on the development of obesity is needed to determine the best allocation of resources for prevention and treatment.

For centuries humans struggled with food scarcity, leaving little opportunity to be in energy surplus (Koning et al., 2008). However, today at a societal level, the contemporary environment provides a food-landscape with 24-hour access to food. The low cost of commodity crops and global trade has resulted in changing food supplies, allowing many countries, including NZ, access to an abundance of highly palatable, inexpensive, ultra-processed food that is energy rich yet nutrient poor (Luiten et al., 2016). Low income countries also suffer from poor food availability and thus lack of dietary diversity, leading to overconsumption of starchy staples (Steyn and Mchiza, 2014). This has likely contributed to an obesity epidemic that would otherwise not have been seen in low income countries, with the underlying issue being over-consumption of energy. Whilst consumption of simple sugars and some saturated fatty acids have been shown to increase BF%, protein and fibre consumption appear to have positive effects on modulating satiety and energy metabolism (San-Cristobal et al., 2020).

Further, industrialisation, improved transportation and labour-saving devices have led to a more sedentary lifestyle (Ng et al., 2009, Monda et al., 2007, Abubakari et al., 2009, Church et al., 2011). While leisure-time PA appears to be increasing, occupational PA has reduced as a result of machinery replacing manual labour (Knuth and Hallal, 2009, Hallal et al., 2012). Consequently as a population, our overall energy expenditure has reduced and our vulnerability to obesity has increased. This is noticeable in the increased prevalence of obesity among women following their increased presence in the workforce during the 1970's (Church et al., 2011). Current PA guidelines include promoting ≥30 minutes of moderate (or greater) intensity activity (e.g. brisk walking or running) on at least five days of the week (U.S. Department of Health and Human Services, 2018, Ministry of Health New Zealand, 2020). However a 2018 review including data from across 168 countries and including 1.9 million participants, found the global age standardised prevalence of insufficient PA to be nearly one third (27.5%), and women had a higher prevalence compared to men (31.7% and 23.4% respectively) (Guthold et al., 2018). In NZ, only 49% of women met PA recommendations in 2020/21 (Pacific and NZ European women 44% and 51% respectively) (Ministry of Health New Zealand, 2021). In agreement with global trends, NZ women were less physically active when compared to NZ men (57% met PA recommendations) and NZ adults living in the most deprived areas are least likely to meet these PA guidelines (Ministry of Health New Zealand, 2021). Evidence from research studies indicate that total PA volume accumulated throughout the day, regardless of intensity, may be as important as time spent in the intense moderate-to-vigorous PA (MVPA), for lowering markers of metabolic disease risk (e.g., fasting insulin, high density lipoprotein (HDL)) (Ekelund et al., 2007, Swindell et al., 2018). Further, there is growing concern for the impact of sedentary behaviour (SB) on metabolic health, independent of PA. Sedentary behaviour defined as low energy expenditure (≤ 1.5 metabolic equivalents (METs)) in a sitting or reclining posture during waking times, has been linked to obesity and poor metabolic health (Tremblay et al., 2017, Proper et al., 2007). In particular, SB adversely impacts on general cardiometabolic health (Chastin et al., 2015, Brocklebank et al., 2015), diabetes, cardiovascular disease, some cancers and all-cause mortality (Wilmot et al., 2012) (Ekelund et al., 2016) (De Rezende et al., 2014, Lynch et al., 2018). Accelerometery data from a 2018 review indicated that adults spend approximately 8.2 hours per day being sedentary (Matthews et al., 2008, Bauman et al., 2018). It has also been suggested that not only the overall quantity of SB is detrimental to cardiometabolic health, but also the nature in which it is accumulated. A less prolonged sedentary accumulation pattern (i.e., more regular breaks, shorter sedentary bouts) has been associated with lower body mass index (BMI) (Chastin et al., 2015),

improvements in postprandial glucose metabolism (Saunders et al., 2018), and all-cause mortality (Diaz et al., 2017). However, results have been inconsistent with some studies showing no association between breaks in SB or in sedentary bout duration and all-cause mortality (Jefferis et al., 2019).

Minority groups, including indigenous populations in Europe and immigrants from low and middle income countries (Langøien et al., 2017), as well as Pacific women in NZ (O'Brien et al., 2022) had higher levels of SB; however, research comparing associations between SB and cardiometabolic health across ethnic groups is scarce (Nagy et al., 2019, Brodersen et al., 2007).

Increasingly, evidence suggests that microbial diversity and functionality in the gut may play a critical role in obesity by modifying energy extraction from food, specifically, breaking down non-digestible substrates like dietary fibres and producing short chain fatty acids (SCFAs); These SCFA's, such as butyrate, have regulatory functions in anti-inflammatory and immune response, lipid, cholesterol and glucose metabolism as well as gut barrier integrity (Fart et al., 2020).

A healthy microbiota can be described as increased richness and diversity as well as certain compositional (bacterial phyla, genera, family taxa) and functional features (metabolic properties). It has been suggested that microbial dysbiosis, including a reduction in microbial diversity, such as the loss of beneficial bacteria (e.g. *Bacteroidetes)* and butyrate producing bacteria (e.g. *Faecalibacterium*), is a contributing factor in the development of a number of diseases, including obesity and T2D (Hartstra et al., 2015, Bäckhed et al., 2004, El-Jurdi et al., 2017, Turnbaugh et al., 2009).

Considering a major function of the gut microbiota is to ferment non-digestible substrates, diet is considered the main extrinsic factor that can alter the microbiota (Cronin et al., 2021). Physical activity has been shown to promote the production of gut microbiota metabolites, like SCFAs (Clark and Mach, 2017), through increased relative abundance of SCFA producing species (Brahe et al., 2015). PA has also been shown to reduce pro-inflammatory cytokines and oxidative stress in the gut and reduce the bowel transit time (Shahar et al., 2020).

This thesis is part of a wider study called the PRedictors linking Obesity and the gut MIcrobiomE (PROMISE) study. The PROMISE study is the first to characterise the gut microbiota in two population groups of women, with markedly different metabolic disease risk (Pacific and NZ European) and different body fat profiles (normal and obese). The

PROMISE study was designed to test the primary hypothesis that reduced gut microbiota diversity, is a key biological driver of obesity and unhealthy body fat distribution in women, whereas greater gut microbiota diversity and gene richness is protective. The secondary hypothesis is that differences in diet, taste perception, sleep, and in the case of this thesis PA and SB affect the associations between the gut microbiota, metabolic regulation, and body fat profiles.

1.1 Justification of the research; Aim and objectives

A more comprehensive understanding of the link between PA, SB and diet, and their association with obesity, metabolic health and the gut microbiota will enhance our understanding of the aetiology of obesity. Better understanding of the aforementioned relationships will help to inform public health guidelines and clinical treatment options to help to curb the obesity epidemic and improve public health. Researching women allows this research to focus on the gender with highest obesity rates but lowest participation in PA, therefore knowledge gained will have the ability to make a greater impact. Additionally, it will assist in increasing knowledge to reduce the risk of obesity across generations. By focusing on Pacific (70 % obesity prevalence) and NZE women (30 % obesity prevalence) we will gain valuable insight into groups who differ in metabolic disease risk as well as socio-economic and ethnic-cultural characteristics. Furthermore, recruiting normal and obese BMI participants, this research will investigate the greatest difference in body composition and therefore may increase the chance of observing associations between PA, SB and metabolic health markers including the gut microbiota, that are associated with obesity.

Before I investigated the associations between PA and the gut microbiota (chapter 7) the research group felt it was important to first investigate and understand how physically active and/or sedentary our population was, and to what extent these behaviours were associated with metabolic health and body composition (chapter 4 and 5 respectively). Further, because of the known association between diet and gut microbiota we felt it was important to determine if there was any association between dietary nutrient intake and PA to help guide the statistical approach when investigating PA and the gut microbiota (chapter 6). Evidence from research studies indicate that total PA volume accumulated throughout the day, regardless of intensity, may be as important as time spent in the intense moderate-to-vigorous PA (MVPA), for lowering markers of metabolic disease risk. For much of the population, SB or light PA occupy most of their waking hours, therefore, the WHO guidelines of at least 150 minutes of MVPA per week may be out of reach for many populations. An ultimate goal of

this work was to help inform lifestyle (physical activity) guidelines, therefore we wanted to investigate if there is any benefit in encouraging total PA, independent of intensity, in high-risk groups.

Therefore, the **aim** of this research was to describe the PA and SB, and to explore the associations of PA and SB with body composition, dietary intake, biomarkers of metabolic health, and gut microbiota composition of two groups of healthy women aged 18-45 years; Pacific and NZE, with different metabolic disease risk and different body fat profiles (lean and obese). The scope of this thesis is outlined in Figure 1.1.

Objective 1: To determine if objectively measured PA is associated with different metabolic health risk and body composition and whether these associations differ between ethnic groups.

H^1: *We hypothesise that increased PA (MVPA, light and total PA) would be associated with lower metabolic risk and lower BF%, and that these associations would not differ between ethnic groups.*

Objective 2: To determine if objectively measured SB is associated with different metabolic health risk and body composition and whether these associations differ between ethnic groups.

H^2: *We hypothesise that increased time spent in SB, and prolonged bouts of SB would be associated with increased BF% and higher metabolic risk, and that these associations would not differ between ethnic groups.*

Objective 3: To explore associations between meeting the PA guidelines and nutrient intakes and whether this differs between ethnic groups.

H^3: *We hypothesise that meeting the PA guidelines will be associated with a healthier diet from a nutrient perspective (higher percentage of participant meeting AMDR's and higher micronutrient intakes) compared to not meeting the PA guidelines, and that this would not differ between Pacific and NZE women.*

Objective 4: To explore the possibility that PA and SB (MVPA, total PA, sedentary behaviour and/or meeting PA guidelines *versus* not meeting PA guidelines) are associated with broad

taxonomic composition (family level) and α-diversity of gut microbiota that could drive beneficial metabolic changes to human hosts.

H^4: We hypothesise that increased time spent in PA, reduced time spent in SB and meeting the PA guidelines will be associated with increased α-diversity and higher relative abundance of health promoting bacteria (family level) in the human gut.

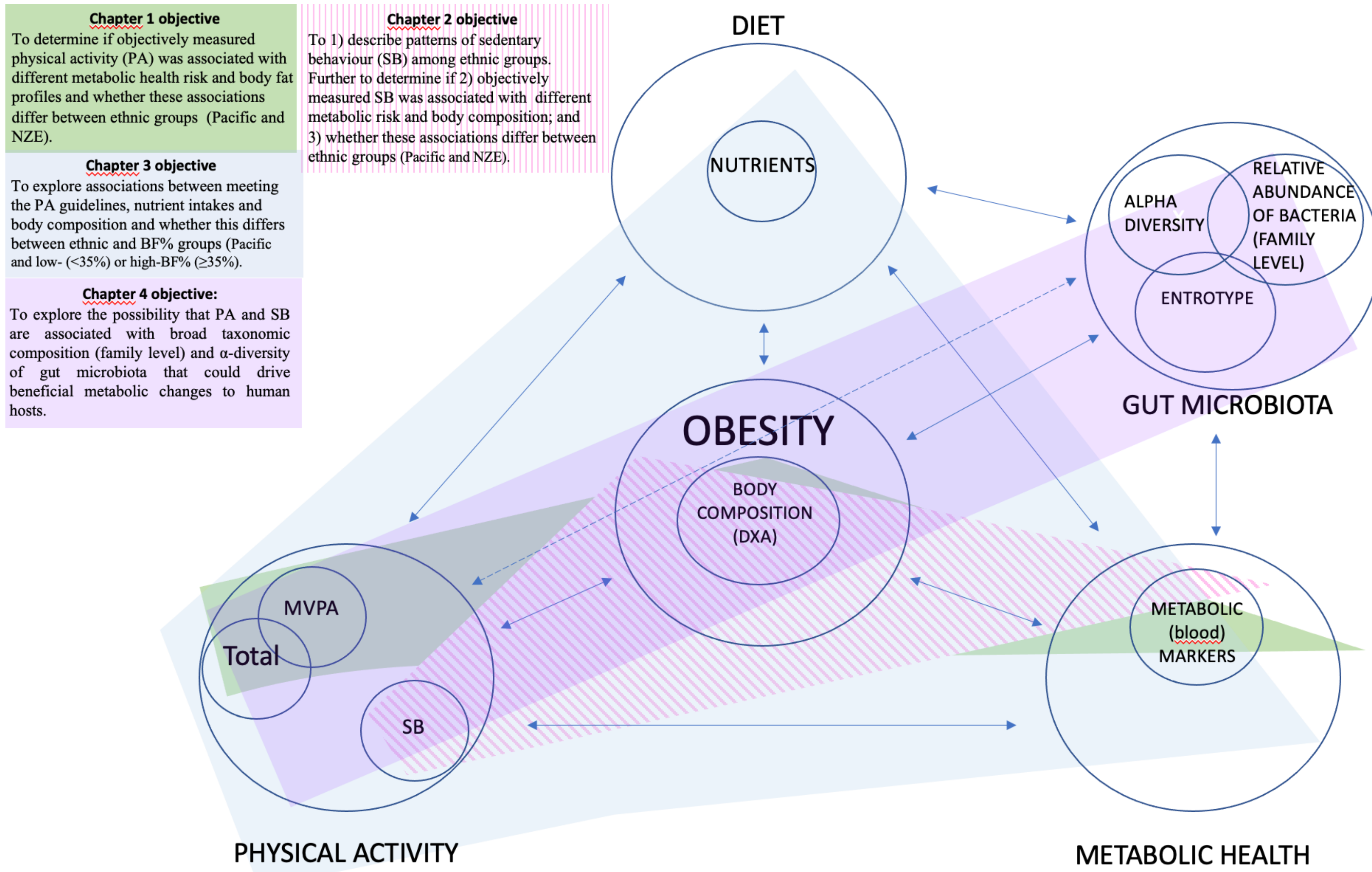

Figure 1.1 Thesis scope

1.2 Structure of the thesis

This PhD thesis begins with a review of the literature (chapter 2) which presents the public health concern of obesity and its association with PA and SB. The review discusses PA and SB assessment and concludes with discussion of the association between obesity and the gut microbiota and the role PA may play in this relationship.

A methodology chapter (chapter 3) then outlines the studies general methods. This PhD thesis is written in the style of a PhD thesis by publication, therefore the methodology chapter is followed by four results chapters (chapters 4-7), written in manuscript form. These four research chapters address the aforementioned aims and objectives of the thesis. Chapter 4 explores the relationship between PA, body composition and metabolic health among Pacific and NZE women. Chapter 5 then explores the relationship between SB, body composition and metabolic health among Pacific and NZE women. Chapter 6 investigates associations between dietary nutrient intake and meeting the PA guidelines. The final research chapter then explores associations between PA, SB and the gut microbiota among Pacific and NZE women. The main results are then discussed together in the final discussion chapter (chapter 8), bringing to light their combined public health significance. Methodological strengths and limitations are also discussed, and the thesis concludes with recommendations for future research. Table 1.1 outlines the contributions by each researcher involved in this PhD study.

Table 1.1 Contributions by each researcher involved in this PhD study

Researcher	Contribution
Joanne Slater **PhD researcher**	• Recruited participants and involved in the organisation and data acquisition of the PROMISE study. • Conducted food record reviews and processed dietary data for the PROMISE study. • Processed all the physical activity, sedentary behaviour and PSQI sleep data. • Analysed all physical activity, sedentary behaviour, sleep (PSQI) and dietary data. • Interpreted results. • Responsible for all aspects of the manuscripts involving conceptualisation and design of manuscripts, literature search, drafting, editing and submission of manuscripts. • Main author of all four manuscripts.
Professor Rozanne Kruger **Primary supervisor**	• Conceptualisation and design of the Promise study, acquisition of funding, supervision of data collection and processing. • Mentorship, connecting PhD candidate with key collaborators. • Reviewing of thesis. • Conceptualisation and design of all four manuscripts. • Revised manuscripts for all four research chapters.
Professor Carol Wham	• Reviewing of thesis. • Mentorship, connecting PhD candidate with key collaborators. • Conceptualisation and design of all four manuscripts. Revised manuscripts for all four research chapters.
Associate Professor **Jennifer Miles Chan**	• Reviewing of thesis. • Mentorship, connecting PhD candidate with key collaborators.

Researcher	Contribution
	• Conceptualisation and design of all four manuscripts. Revised manuscripts for all four research chapters.
Professor Bernhard Breier	• Conceptualisation and design of the Promise study, acquisition of funding and human ethics approval, supervision of data collection. • Conceptualisation and design of Chapter 4. Revised Chapter 4.
Dr Wendy O'Brien	• Assisted with the design of the accelerometer protocol. • Revised Chapter 4.
Dr Marine Corbin	• Assisted with statistical analyses and interpretation of results for all four research chapters.
Professor Jeroen Douwes	• Conceptualisation and design of the Promise study, acquisition of funding Assisted with statistical analyses and interpretation of results for all four research chapters.
Emeritus Professor Gerald Tannock	• Conceptualisation and design of the Promise study, acquisition of funding. • Processed and analysed microbiota data, assisted with interpretation of results for Chapter 7. • Revised manuscript four.
Dr Blair Lawley	• Processed and analysed microbiota data.
Dr Nikki Renall (PhD candidate for the Promise study at the time)	• Recruited participants, involved in the organisation and data acquisition of the PROMISE study. • Conducted food record reviews and processed dietary data for PROMISE study. • Revised Chapter 6.

Researcher	Contribution
Dr Sophie Kindlesides (PhD candidate for the Promise study at the time)	• Recruited participants, involved in the organisation and data acquisition of the PROMISE study.
Niamh Brennan	• Study co-ordinator. • Recruited participants, involved in the organisation and data acquisition of the PROMISE study.
Moana Manukia, Owen Mugridge, Shakeela Jayasinghe, Sherina Holland, Marilize Richter, Beatrice Drury, Laura Mickleson, Bronte Anscombe, Elizabeth Cullen, Guojiao (Maggie) Cao, Ashleigh Jackson, PC Tong	• Research assistants.
Wendy O'Brien	• Openly shared knowledge in accelerometer data processing.

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Chapter 2: Literature review

2.1 The current obesity situation

2.1.1 Obesity Definition

Obesity is an abnormal or excessive accumulation of fat that presents a risk to health (WHO, 2016). It effects people of all ethnicities all around the globe, and is one of the most prominent public health concerns of the twenty-first century. Obesity is a highly prevalent nutritional and metabolic disorder, one of the major risk factors contributing to the development of non-communicable diseases (NCD's) (World Health Organisation 2021). Obesity is caused by a persistent energy intake in excess to our metabolic needs.

2.1.2 Worldwide and NZ prevalence of obesity

Obesity prevalence is at an all-time high. Across the Organisation for Economic Co-operation and Development (OECD) countries the most recent data estimated that according to body mass index (BMI), 19.5% of the adult population were obese (BMI ≥30.0 kg/m^2) (Devaux et al. 2017), and more than one in two adults in the OECD were classified as overweight or obese (BMI >25.0 kg/m^2) (Devaux et al. 2017).

Alongside other Pacific nations, New Zealand (NZ), has some of the highest obesity rates in the world (OECD 2017). In 1977, according to BMI, 10% of adults living in NZ were considered obese, this figure tripled, just over 40 years later (2020/21) with 34.3% of NZ adults (≥15years) being classified as obese (BMI ≥30 kg/m^2, or International Obesity Task Force equivalent for 15-17 years; BMI > 2 standard deviations above the WHO growth standard median) (Ministry of Health New Zealand 2021). In developed countries, obesity is predominantly observed in socioeconomically disadvantaged populations (Zhang and Wang 2004) and among women (GBD 2015 Obesity Collaborators 2017; NCD Risk Factor Collaboration 2016; The GBD 2015 Obesity Collaborators 2017). Obesity also disproportionally effects some ethnic groups (Hales CM 2020), as is the case in NZ. NZ adults living in the most deprived areas are 1.6 times more likely to have obesity compared to adults living in the least deprived areas (Ministry of Health New Zealand 2021). Obesity prevalence is highest among NZ Māori (the indigenous population of NZ) (52.9%) and Pacific (people who identify with a Pacific (eg Samoan, Tongan, Fijian) with or without other ethnicities, residing in NZ) (Stats New Zealand 2015) (74.3%) women when compared to NZ European (NZE) women (33.5%) (BMI ≥30 kg/m2 or International Obesity Task Force equivalent for 15-17 year olds) (Ministry of Health New Zealand 2021).

2.1.3 Consequences of obesity - metabolic disturbances

Individuals present along a spectrum ranging from a healthy state, to a chronic pro-oxidant, pro-inflammatory state which leads to deterioration of glucose and lipid metabolism (Kaur 2014), to a state of metabolic dysfunction, such as type-2 diabetes (T2D), either insulin or non-insulin dependent. Somewhere along this spectrum there is an altered metabolic state, which may progress and culminate in ß-cell failure, hyperinsulinemia, hypertriglyceridemia, glucose intolerance and hypertension. This places the individual at heightened risk of several co-morbidities; cardiovascular disease (CVD), some cancers, as well as a heightened risk of all-cause mortality (Arnold et al. 2016; Aune et al. 2016; The GBD 2015 Obesity Collaborators 2017).

Insulin, the major metabolic hormone that largely regulates glucose metabolism, is released after eating. In response to eating/ increased blood glucose levels, ß-cells in the pancreas are stimulated to release insulin, which in turn stimulates receptors on various cell membranes (skeletal, adipose, liver, neural) to facilitate the uptake of blood glucose into cells to be used and/or stored as energy. As glucose from the blood is transported into the cells, ß-cells are no longer activated and insulin levels decrease. However, for obese individuals there may be constantly raised blood glucose levels (in part, due to excess adipose tissue being mobilised resulting in FFA release into the system). Hypertriglyceridemia, through various biochemical steps, leads to FFA "*competing*" for uptake with glucose (from intercellular to intracellular spaces), leaving blood glucose levels higher, which in turn leads to constant increased stimulation of pancreatic ß-cells and subsequent increased levels of plasma insulin (Bagdade et al. 1967). The body may cope initially, however over time, with the chronic stimulation of ß-cells and therefore raised insulin levels, the insulin receptors/on the cell membranes become less effective/ sensitive, less glucose transportation molecules are placed into the cell membrane, and the individual remains in a state of hyperglycaemia. Longer-term, the ß-cells, after being chronically stimulated by increased blood glucose, may 'wear out' and stop producing adequate insulin to reduce blood glucose. Chronic hyperglycaemia is detrimental to well-being as it promotes formation of advanced glycosylated end-products (AGE's) which lead to cellular damage (neuropathy, vascular degeneration etc.), as well as increased inflammatory mediators (e.g. IL-6, TNF-α and C-reactive protein (CRP)) which may also contribute to microvascular damage, and culminate in atherosclerosis.

Whilst insulin acts to directly regulate blood glucose once food is ingested, other hormones such as leptin and ghrelin, help regulate appetite and satiety. Leptin, a hormone secreted by

adipocytes in proportion to body fat stores, acts on the hypothalamus, to reduce appetite indicating the body is sufficiently fuelled and there is no need for additional energy intake and to use energy as required. Not surprisingly, leptin levels are reasonably insensitive to a single meal ingestion as adipose storage and lipid metabolism is as not as finely-tuned as glucose metabolism (Polonsky et al. 1988). Some research supports the thought that much like insulin, obese individuals experience a down regulation/ reduced sensitivity of leptin receptors, consequently, although circulating leptin levels are often very high in obese individuals they become less sensitive to the hormone and therefore less effective at appetite suppression (Izquierdo et al. 2019). This is termed “leptin resistance”. On the other hand, ghrelin, which is primarily released by cells in the stomach tissue, trigger increased appetite with circulating blood levels correlating well with energy intake. Interestingly, in obese individuals, ghrelin levels do not seem to reduce as significantly following a meal, when compared with non-obese (Le Roux et al. 2005). It is important to note however that the relationship between leptin, ghrelin, body fat and dietary intake is complex, and there are many additional factors that influence hormone levels (e.g. sleep (duration and quality) and stress).

Therefore, fasting levels of insulin are a suitable surrogate measure of peripheral insulin sensitivity (McAuley et al. 2001). Of note, insulin sensitivity is related to both total body adiposity and where that fat is distributed, a key determinant of reduced insulin sensitivity being visceral fat (Porte Jr et al. 2002). The metabolic consequences of obesity are strongly correlated with body fat in a central or upper body (android) distribution (Ibrahim 2010). Increased fat in the abdominal cavity or visceral fat is associated with increased pro-inflammatory markers (IL-6, TNF-α and C-reactive protein) and reduced levels of anti-inflammatory markers (adiponectin), indicating a pro-inflammatory environment that can lead the aforementioned metabolic disturbances (Ibrahim 2010).

2.1.4 Measurement of body fat and classification of obesity

It is difficult to accurately measure total fat in the body, as all measurement techniques rely on assumptions including density of body tissues or body concentration of water and/or electrolytes and therefore have an element of error (Duren et al. 2008). Whilst these assumptions are relatively well validated in a “normal” body fat population, the same validation has not been undertaken in obese individuals (Duren et al. 2008). There are various techniques, direct and indirect, which are used to estimate body composition and these all have various benefits and limitations (Table 2.1). A wide variety of BF% cut-off points have been used to define obesity, varying between 30- 37% for women, and 20-30% for men (Dickey et al. 1998;

Oliveros et al. 2014) (Fitch and Bays 2022). Most recently the Obesity Medicine Association classified obesity as >35% body fat for adult women and >30% for adult men (Fitch and Bays 2022). Body mass index and waist circumference (WC) are simple, quick, non-invasive and inexpensive measures, they are commonly used in both research and clinical practice. At a population level BMI is a reasonable body composition measure, but it is less appropriate when comparing ethnicities for whom the relationship between BMI and body fat % varies; as is the case for Caucasian and Polynesian populations (Hunma et al. 2016; Rush et al. 2007). Similarly, WC is investigator-dependent, this limitation can be minimised by having only one investigator taking WC measurements, or measurements of inter (and intra) investigator variability being collected (World Health Organisation 2008).

Table 2.1 Measurement of body fat and classification of obesity

Measure	Description	Equipment required	Strengths	Limitations	Classification of obesity
Body mass index (BMI) or Quatelet's Index	Ratio of height to weight squared. Calculated as weight (kg)/height^2 (m) (Keys et al. 1972).	Scales Stadiometer	Simple, quick, non-invasive, cost-effective. At a population level correlates closely with the degree of body fat.	Less appropriate when comparing ethnicities for whom the relationship between BMI and body fat % (BF%) varies (Hunma et al. 2016; Rush et al. 2007). Inappropriate when using in athletic muscular populations as does not differentiate fat mass from lean body mass (Garrido-Chamorro et al. 2009).	WHO's standardised cut points for; underweight (BMI < 18.5), normal weight BMI ≥18.5 and ≤24.9); overweight (BMI ≥25.0 and ≤29.9) and obesity (BMI ≥30.0) (World Health Organisation 2021).
Air Displacement Plethysmography (ADP)	Measures the displacement of air, and uses an algorithm to determine BF% (von Hurst et al. 2016).	BodPod chamber	Quick, easy, and relatively non-invasive (Harvard School of Public Health 2022).	Expensive (Harvard School of Public Health 2022). Relies on pressure temperature and water/ sweat also humidity in lungs can induce error. Therefore, strict protocols must be adhered to, however, it is difficult to completely eliminate this error.	A wide variety of BF% cut-off points have been used varying between 30- 37% for women, and 20-30% for men (Fitch and Bays 2022; Oliveros et al. 2014).
Bio electric impedance analysis (BIA)	Estimates total body water, fat-free mass, and fat mass by measuring the resistance of the body as a conductor to a small alternating electrical current. The rate which the currents propagate	Bioelectric impedance analyser	Quick, easy to use, non-invasive, portable. Some models have been shown to underestimate fat mass when compared to DXA and ADP (e.g. InBody 230) (von Hurst et al. 2016).	The rate of propagation of the currents is influenced by hydration status which is difficult to control therefore, errors may occur in the results. The ratios of extra and intracellular water is altered in people with obesity, which may	A wide variety of BF% cut-off points have been used varying between 30- 37% for women, and 20-30% for men (Oliveros et al. 2014) (Fitch and Bays 2022).

Measure	Description	Equipment required	Strengths	Limitations	Classification of obesity
	allows calculation of BF %. (Chumlea and Guo 1994; Lukaski et al. 1985).			affect the results. Not well validated in people with obesity or who are overweight (Gray et al. 1989; Kushner et al. 1990). Equations to describe statistical associations are based on biological relationships for a specific population, therefore the equations are only useful for participants that closely match the body composition of the reference population.	
Skinfolds/ calliper testing	Uses callipers to measure skin thickness at various regions of the body, and then an algorithm to calculate BF% (Harvard School of Public Health 2022).	Skinfold callipers metal anthropometric tape measure	Portable, cost effective, quick (Harvard School of Public Health 2022).	Requires a skilled trained operator. Possible examiner error and algorithm limitations. Limited use in overweight people or people with obesity as most skinfold callipers have an upper measurement limit of 45 to 55 mm. In adults, BMI has a stronger statistical relationship between percent or total body fat than that of skinfolds (Roche et al. 1981).	A wide variety of BF% cut-off points have been used varying between 30- 37% for women, and 20-30% for men (Oliveros et al. 2014) (Fitch and Bays 2022).
Dual-energy X-ray absorptiometry (DXA)	Uses a low dose of radiation to assess the density of tissue to quantify fat, lean and bone tissues (Harvard	DXA scanner	Provided the correct protocol is followed, a valid, accurate and reliable method for obtaining an actual measure of total BF% and distribution of fat in the body (von Hurst et al. 2016). It is highly	Insensitive at high and low BF% (Williams et al. 2006). Expensive, requires specialist equipment and an operator. Requires hospital/clinic visits,	A wide variety of BF% cut-off points have been used varying between 30- 37% for women, and 20-30% for men (Oliveros et al. 2014) (Fitch and Bays 2022).

Measure	Description	Equipment required	Strengths	Limitations	Classification of obesity
	School of Public Health 2022). By subdividing the body using specific well-defined cut lines, regional body composition can be measured (von Hurst et al. 2016).		sensitive and user-friendly for the subject and operator.	making it impractical for many (von Hurst et al. 2016). DXA software used to estimate fat and lean tissue is based on assumptions regarding levels of hydration, potassium content, or tissue density, these assumptions vary by manufacturer (Kohrt 1995). Intra- and intermachine differences (Roubenoff et al. 1993). Exposes the patient and operator to very small dose of radiation (von Hurst et al. 2016).	
Waist circumference	Obtained by measuring th e abdomen at one of the following locations, using : - Narrowest part of the waist(Lohman et al. 1988) - Midpoint between the lower margin of the least palpable rib and the top of the iliac crest(World Health	metal anthropometric tape measure	Quick, simple, inexpensive (Harvard School of Public Health 2022).	Investigator-dependent (World Health Organisation 2008).	Average :<94 cm men, <80 cm women. Increased risk: 94-101 cm men, 80-87 cm women. Substantially increased risk: >102 cm men, >88 cm women (World Health Organisation 2008).

Measure	Description	Equipment required	Strengths	Limitations	Classification of obesity
	Organisation 2008) - Immediately above the iliac crest (Wang et al. 2003) - Level of the umbilicus (Rexrode et al. 1998)				

2.1.5 Drivers of obesity

On a simplistic level, a persistent positive energy balance leads to adiposity. In reality, the drivers of having obesity are extremely complex. The Foresight Systems Obesity Map (Figure 1.) demonstrates this complex web of interactions (Vandenbroeck et al. 2007). Populations with increased predisposition to obesity may be explained in part by certain genetic, behavioural and societal (social/environmental) factors.

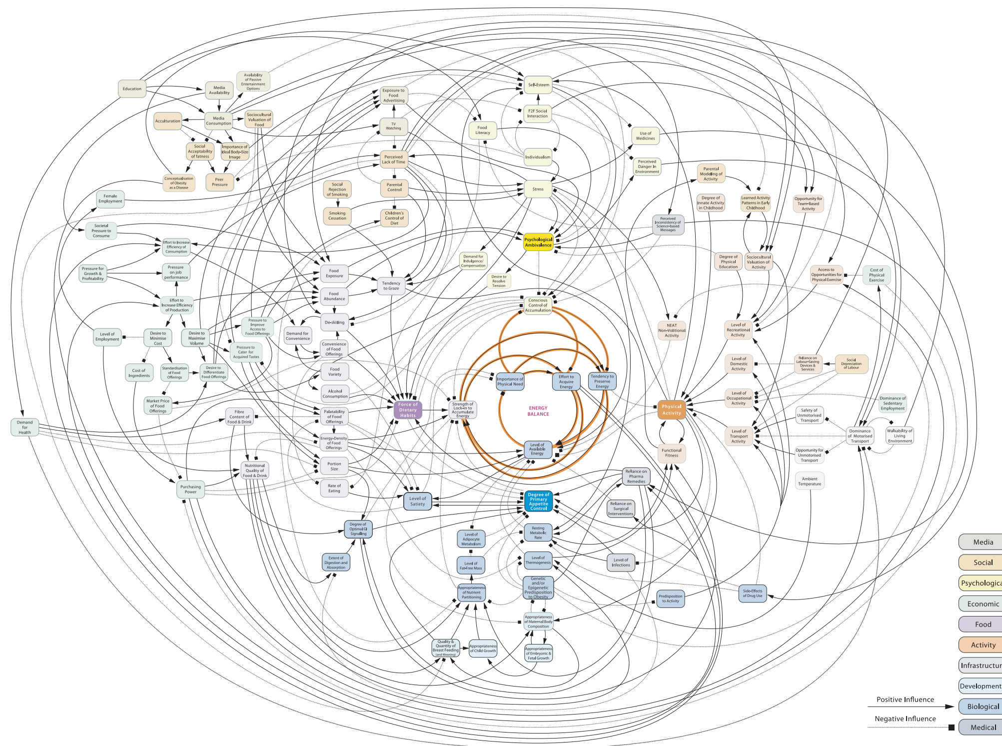

Figure 2.1 Foresight Obesity System Map (Vandenbroeck et al. 2007)

Family history of obesity has been shown to be associated with higher BMI and increased risk of having obesity (Mangla et al. 2019). This association is likely the result of an interaction between genetic traits and environmental factors, such as shared lifestyle characteristics (Corica et al. 2018), which are shared to a larger degree among family than among the general population. Research has shown that "familial aggregation" exist in familial correlations of obese/overweight in childhood, where daughters are more likely than sons to have obesity/overweight if their parents also do (Mangla et al. 2019).

Global and NZ trends in obesity show a significant rise in women when compared to men (The GBD 2015 Obesity Collaborators 2017). Between the 2015/16 and 2020/21 NZ Health surveys obesity prevalence as measured by BMI increased 2.0% (from 30.8% to 32.8%) among men

and 3.2% (32.7% to 35.9%) among women (Ministry of Health New Zealand 2016, 2021). The long-term health impact is alarming, because increased adiposity in women of childbearing age is associated with acute and chronic adverse health outcomes, including the perpetuation of increased obesity (J. Eriksson et al. 2001; J. G. Eriksson et al. 2014) and cardiometabolic disease risk (Hochner et al. 2012) for the next generation. In addition to this female obesity contributes to anovulation and menstrual irregularities resulting in higher risk of infertility (Zain and Norman 2008). The prevalence of central obesity among individuals aged 40 or greater has been shown to be nearly twice that of younger adults aged 15-40 years (48% vs 23.8%) (Wong et al. 2020). A lower basal metabolic rate associated with aging likely contributes to this (Visser et al. 1995). Furthermore, research has shown obesity at age five years to be associated with significantly higher BMI at age 50 years (Rundle et al. 2020). Specifically among women, increased body weight with aging has been reported (T. C. Yang et al. 2017). From puberty through to menopause, fat mass progressively increases. Post menopause, a further increase in fat mass as well as reduction in lean body mass occurs, increasing the prevalence of sarcopenia in women (Franklin et al. 2009; St-Onge 2005).

Obesity disproportionally effects some ethnic groups. The 2017/18 NHANES data showed that in the United States of America (USA), Non-Hispanic Blacks (49.6%) had the highest age-adjusted prevalence of obesity (defined by BMI), followed by Hispanics (44.8%), non-Hispanic Whites (42.2%) and non-Hispanic Asians (17.4%) (Hales CM 2020). In NZ, of particular concern is the higher obesity prevalence observed in minority population groups, including NZ Māori and Pacific peoples domiciled in NZ. When stratifying NZ adult obesity prevalence (defined by BMI) by ethnicity, the adult prevalence rates vary considerably and are associated with substantial health inequalities. Pacific peoples and the Māori population in New Zealand have the highest obesity rates, 71.3% and 50.8% respectively, Asian the lowest (18.5%), and the NZE/Other adult population have 31.9% obesity rates ((N. Z. Ministry of Health 2019; Ministry of Health New Zealand 2021). The reason for this significant difference is unclear but is hypothesised that it may be linked to genetic variance or social/environmental differences between the populations (Swinburn et al. 2011).

Historically considered a disease of affluence, today, obesity is not confined to high income countries (Ng et al. 2014). Many low income countries, including Island nations in the Pacific and Caribbean as well as countries in the Middle East, Africa and Central America, show a steady rise in the prevalence of obesity (NCD Risk Factor Collaboration 2016; Ng et al. 2014) (Chukwuonye et al. 2013). This has resulted in a “double burden of malnutrition” in these

countries, with malnutrition in the form of under nutrition continuing to exist (Ng et al. 2014; World Health Organisation 2017a). In lower-income countries, people with higher socioeconomic status are more likely to be obese, whilst in high-income countries, those with higher socioeconomic status are less likely to be obese (Pampel et al. 2012).

Specifically in NZ, the 2020/21 NZ Health Survey found that after adjusting for age, sex and ethnicity adults living in the most socioeconomically deprived areas were 1.6 times as likely to have obesity as adults living in the least deprived areas (Ministry of Health New Zealand 2021). In NZ, Pacific and Māori people are over represented in areas of high deprivation when compared to NZE (Ministry of Health New Zealand 2021).

Research has shown that a large portion of the association between socioeconomic position and obesity is mediated through health behaviours such as diet and PA (Foster et al. 2018; Pampel et al. 2010; Shaikh et al. 2015). The more deprived one is, the more vulnerable they are to the obesogenic environment, access to food and PA is limited by cost, time and geographical locations. Areas of high deprivation may have smaller tax income to fund recreational PA facilities, whilst simultaneously having higher crime rates (Exeter et al. 2017), a potential barrier to engaging in PA in public spaces .

Demanding social expectations; including shift work, travel between time-zones, time spent in artificial light and the use of electronic media all contribute to altered sleep patterns (particularly short sleep and shifting sleep timing between weekdays and the weekend).

Our circadian rhythm and sleep homeostasis are the two biological systems that work together to control duration, timing and quality of sleep. Circadian rhythms help to initiate physiological processes and behaviours at appropriate times of the day or night and are near 24 hr oscillations found in all living things; animals, plants and even microbes (Gander 2003). Circadian rhythm misalignment, such as during shift work or jet lag, has been connected to greater risk of metabolic diseases including obesity and T2D (Sebti et al. 2022). Sleep habits are well ingrained by the time we reach adulthood (Miller and Cappuccio 2007). When circadian misalignment occurs, individuals carry out certain behaviours such as sleeping and feeding at times mal-aligned with their circadian rhythm. Misalignment of our natural sleep circadian rhythm leads to a temporary increase in cortisol, increased blood pressure, reduced insulin sensitivity and increased blood glucose levels which are all associated with poor metabolic health (Scheer et al. 2009). Adding to this, sleep deprivation may lead to increased desire for carbohydrate rich and energy dense foods, whilst the feeling of fatigue leads to reduced desire

for PA (Sullivan 2016), reiterating the complex relationship diet, PA and sleep have with one another.

2.2 Physical activity and sedentary behaviour

The health benefits of PA have been promoted throughout Western history. In 400 BC the "Father of Medicine" famously stated "eating alone will not keep a man well, he must also take exercise" (Hippocrates, *Regimen*, *ca* 400 BC), however it wasn't until millennials later, the accumulation of scientific research supported these thoughts. The seminal work of Morris and colleagues investigated men in occupations that required them to routinely engage in aerobic activity (double decker bus conductors, postmen) and compared them to men in sedentary work (bus drivers, clerks), they distinguished that aerobic activity helps to protect against coronary heart disease (Morris et al. 1953). We since know increased PA also reduces the risk of other major chronic diseases including T2D and some cancers (Anderson and Durstine 2019).

2.2.1 Physical activity and sedentary behaviour definitions

Described in Table 2.2, the commonly used classifications of PA intensity are presented, including 'sedentary', 'light', 'moderate' and 'vigorous' intensities. It is well established that PA performed at both moderate and vigorous intensity improve health, therefore these intensity categories are often combined and referred to as moderate to vigorous PA (MVPA) (World Health Organisation 2020a). Typically levels of PA are expressed as metabolic equivalents (METs). One MET is considered the resting metabolic rate or the energy cost of a person at rest (Ainsworth et al., 2000). Therefore, when compared to sitting resting (<1.0 METs), a person's calorie consumption is six times higher when engaging in vigorous PA (>6.0 METs) (Table 2.2). Another way researchers express levels of PA or PA intensity is via perceived exertion where individuals subjectively rate how hard they feel their body is working. The most common tool of this kind being Borg's Rating of Perceived Exertion (RPE) scale (Borg 1970) (Table 2.2). The RPE is a 10-point Likert scale with verbal descriptors, allowing individuals to subjectively rate their level of exertion during PA or exercise testing.

Table 2.2 Physical activity definitions and terminology

Term	Definition
Physical Activity (PA)	Any movement of your body produced by skeletal muscle that results in an increased use of energy. This can include leisure activities as well as work (Caspersen et al. 1985; World Health Organisation 2022).
Exercise	A sub-category of PA that is planned, structured, repetitive, and purposeful in the sense that the improvement or maintenance of one or more components of physical fitness is the objective (Caspersen et al. 1985).
Physical inactivity	Failure to meet PA guidelines (Tremblay et al. 2017).
Sedentary behaviour (SB)	Waking activities performed whilst sitting, reclining or lying, with energy expenditure ≤1.5 metabolic equivalents (Tremblay et al. 2017).

Table 2.3 Defining levels of physical activity intensity

PA intensity	METs (Ainsworth et al. 2011)	RPE (Borg 1970)	Subjective description	Real life examples (Ainsworth et al. 2011)
Sedentary	1.0-1.5	<8	Activities with energy requirement little greater than resting (Tremblay et al. 2017).	Desk work, eating while sitting, reading, sitting watching TV
Light	1.6–2.9	8-10	Activities that feel like you can maintain for hours, easy to breathe and can hold a conversation at the same time (Borg 1970).	Light walking, gardening
Moderate	3.0–5.9	11-13	Activities allowing you to talk a short conversation but not sing (National Center for Chronic Disease Prevention and Health Promotion 2022).	Vacuuming, brisk walking
Vigorous	≥6.0 6.0 -8.9	≥14 14-17	Unable to say a few words without pausing for a breath (National Center for Chronic Disease Prevention and Health Promotion 2022).	Tennis, running
MVPA	3.0-8.9	11-17	Activities ranging from moderate to vigorous intensity.	See moderate and vigorous examples

PA; physical activity, MET's; metabolic equivalent of tasks, RPE; rating of perceived exertion, Borg's scale (6-20) (Borg 1970), MVPA; moderate to vigorous physical activity.

Although "PA" and "exercise" are often used interchangeably, they are not synonymous (Table 2.3). The WHO defines PA as any movement of your body produced by skeletal muscle that results in an increased use of energy (World Health Organisation 2022). This can include leisure activities as well as work (Caspersen et al. 1985). PA can be sub-divided into categories such as work tasks, household tasks, sports, exercises and other activities. Thus exercise is in-fact a sub-category of PA. Exercise is planned, repetitive, purposeful PA someone engages in with the intention of improving fitness or general wellbeing (Caspersen et al. 1985).

It is also important to differentiate between physical inactivity and sedentary behaviour (SB) (Table 2.3). These terms are also not interchangeable. There is no universally agreed definition for SB however in 2012 the Sedentary Behaviour Research Network (SBRN), a group of researchers and health professionals with a strong interest in SB, congregated to address the issue. The following definition was agreed on: "any waking behaviour characterised by an

energy expenditure of <1.5 METs while in a sitting or reclining posture" (Network 2012). This definition has been widely accepted, but SB has also been described as "low level activity with energy expenditure only marginally above resting" (Pate et al. 2008) and "activity equivalent to sleeping, sitting, lying or non-upright activities" (Tremblay et al. 2017). Most research studies define physical inactivity as an absence of PA (MVPA), or not meeting PA guidelines (Network 2012). However it has also been described as "not engaging in any PA and/or being in the lowest category of PA" (Sharara et al. 2018). Unless otherwise specified, the definitions used in this thesis from here on in are outlined in Table 2.3.

The scientific community agrees that SB's include watching television, sitting, lying down, reading, screen-based activities, driving a vehicle or using a computer (Edwardson et al. 2012) being low exertion activities typically ranging between 1.0-1.5 METs (Pate et al. 2008) (Table 2.3). Although the SBRN agreed upon a definition of SB in 2012, the integral details on the components of SB were still ambiguous at this time. Including the thought that sitting and lying are more detrimental to health when compared to standing. However, with the aforementioned definition "neither standing nor sitting can adequately be described just in terms of energy expenditure or neuromuscular activity", therefore this definition doesn't allow distinction between the two behaviours (Viir and Veraksitš 2012). This is important as it dismisses the effect of gravity on the muscles holding one's body upright (Viir and Veraksitš 2012). Also, a review by Hamilton et. al. proposed an animal model-based biological mechanism, suggesting prolonged sitting impairs lipoprotein lipase activity and this could be prevented by changing posture to standing, with no further benefit from exercise, suggesting standing is not a sedentary activity (Hamilton et al. 2007).

The SBRN re-convened in 2017, to establish consensus definitions for terms relevant to SB research and to develop a conceptual model to illustrate the hierarchical structural connections among the various terms (Tremblay et al. 2017). This consensus statement allows for clear, common and accepted terminology and definitions within the area of SB research. A thorough outline of these definitions can be found in the consensus statement, however key changes/updates included the addition of the term "lying" to the aforementioned definition of SB. They also introduced the term "stationary behaviour" , allowing "standing" to be appropriately classified. They provided a clear definition of "passive standing" (≤2.0 METs) and "active standing" (>2.0 METs) and suggest researchers use the term "stationary time" to report data gathered from accelerometers that do not measure posture. The definitions in the consensus statement clarify the distinction between sedentary "behaviours" (with context) and

“time” at a determined level of intensity (without context, which includes most accelerometer and inclinometer data). They also proposed a standardised approach for defining bouts and interruptions/breaks; with sedentary bouts defined as “a period of uninterrupted sedentary time” and sedentary interruptions/breaks defined as “non-sedentary bout in between two sedentary bouts”. Although the research community still lacks a clear definition of the minimum duration of a sedentary bout that could be deemed as prolonged sedentary time. The SBRN agreed with Kim et al., (Kim et al. 2015) that a sedentary bout threshold of 10 minutes would be a conservative definition to capture the prolonged nature of SB. Further, the conceptual model (Figure 1) was created to provide researchers investigating relationships between, and among, the various movement behaviours throughout the entire day (i.e., sleep, SB’s and various intensities of PA) with clarity on definitions of terminology related to SB.

Figure 2.2 Illustration of the final conceptual model of movement-based terminology arranged around a 24-h period, taken from "Sedentary Behavior Research Network (SBRN) – Terminology Consensus Project", by Mark S. Tremblay et al., 2017 IJBNPA. 14(1), 7.

2.2.2 Measurement of physical activity and sedentary behaviour

Like all human behaviours, the measurement of PA is complex. All measurement techniques have inherent strengths and limitations (Table 2.4). Subjective measures of PA are commonly used due to being comparatively practical, inexpensive and easy to use. Self-report methods often rely on memory and require participants to have good literacy skills to accurately record their activities. Questionnaires, for example, are quick, cheap and have a low participant and

researcher burden, although they are vulnerable to recall bias (Dishman et al. 2001). In contrast, self-report PA diaries and logs require participants to record physical activities in real time, eliminating the problem of recall bias (Dishman et al. 2001). They also allow for description of the type of PA the individual is engaging in. However, using self-report methods to differentiate between PA types can be challenging, and thus researcher intervention is often required to gain accurate information. In particular, dissecting out 'sedentary' activities from 'light' activities can be problematic, as the participant would need to have a sound understanding of the difference between the two.

Whilst subjective measures of PA provide useful information on the context of PA, objective measures allow for more reliable estimates of volume and/or intensity of PA. Objective data collection also eliminates recall bias. The doubly-labelled water (DLW) method is considered the gold standard for assessing total daily energy expenditure, due to its high degree of accuracy (Westerterp 2017) (Table 2.4). However, due to its substantial cost, it is rarely feasible. Additionally, DLW does not provide the integral details on daily PA behaviour required, such as duration, intensity or type of PA performed. Nor can it differentiate between the different components of total energy expenditure (i.e., resting energy expenditure, PA energy expenditure, thermic effect of food) (Pedišić and Bauman 2015) and, therefore, the choice of DLW to measure PA is limited to questions relating to overall energy expenditure over a number of days.

As well as becoming increasingly technologically advanced and cost-effective, accelerometers provide valuable, real-time information on PA and SB patterns. This includes duration, quantity and intensity (D. R. Bassett and John 2010). Objective measures, like accelerometery, provide no context of the performed PA, including domain or behaviour type (e.g. driving, working, TV/computer use), therefore they are best used in conjunction with a PA diary/ log to determine this information. Further, simply due to the act of wearing a PA monitoring device, some individuals change their PA behaviours, particularly on the first day of monitoring (Baumann et al. 2018). This is known as "measurement reactivity" and threatens the validity of the data (Baumann et al. 2018). Some studies exclude data from the first and last days of monitoring due to possible incomplete data on these days and to eliminate this potential bias, it also means all participants have the same start time, regardless of the time of day the accelerometer is fitted. However, recently Ullrich et.al. (2021) found only very small shifts in LPA and SB over a 7-day period due to decreased wear time.

In addition, simple advice to participants, reiterating the importance of constantly wearing the accelerometer in order to obtain valid data is important (Baumann et al. 2018).

Participants are either asked to wear the accelerometer only during waking hours, or continuously (24-hour protocol), only removing the device for water-based activities. Due to different life schedules, considerable variation in the time of day/night participants start and stop wearing the accelerometer can occur when only asked to only wear during waking hours. Such differences can bias data, particularly if the main research question concerns reporting patterns of sedentary or light PA, particularly as, anecdotally, the very beginning and end of one's day are most likely associated with sedentary or light PA, and thus these behaviours may be underestimated.

Evidence suggests that, to attain valid and reliable estimates of habitual movement behaviour in population studies, measurements should be made over a seven day period, which allows a buffer to compensate for days during which the accelerometer was not worn for a sufficient length of time (D. Bassett et al. 2015; Matthews et al. 2012a; Pedišić and Bauman 2015; Trost et al. 2005). Although there is consistency in the number of data collection days, due to non-compliance, the number of valid days often varies between participants. Leaving researchers with the decision of the minimum number of "valid" days each participant requires for inclusion in analyses. Most studies range from one to seven valid days (Matthews et al. 2012a). A minimum of four valid days is most common, as it has been shown to be comparable to one week and thus a reliable estimate of habitual PA and SB, with minimal participant burden (Donaldson et al. 2016; Matthews et al. 2012a; Pedišić and Bauman 2015; Trost et al. 2005; Tudor-Locke et al. 2012). Some protocols require at least one of these days to be a weekend day (Trost et al. 2005), which allows for possible variation in activity patterns between week and weekend days to be accounted for.

When creating accelerometer derived estimates of PA and SB, researchers are required to make many data processing and analytical decisions, in order to secure the highest possible data quality as well as maintain consistency among studies (Migueles et al. 2017). Firstly, researchers need to define "non-wear" time. Non-wear time is any period of time during data collection that participants are not wearing their accelerometer, including removal for certain activities such as water-based activity (e.g. swimming, showering, surfing) or contact sports, periods when participants forget to reattach their monitor or decide not to wear it. Identifying non-wear time is difficult because long continuous bouts of accelerometer inactivity (or zero

reading) may be true non-wear time, but equally may occur when a study participant is sitting still for prolonged periods or sleeping/ napping with the accelerometer on. A solution to improve accuracy of wear time estimation is using a skin mounted accelerometer that contain an inbuilt skin temperature sensor (e.g. ActivPAL or AX3) (Duncan et al. 2018).

The most frequently used definition of non-wear time being a 60-min criterion allowing for 0–2 minutes of non-zero counts (Colley et al. 2011; Hansen et al. 2013; Spittaels et al. 2012; Troiano et al. 2008). However, some validation studies recommend longer durations of zero accelerometer readings (90–180 min), both with and without allowance of interruptions, to meet the criteria for non-wear (King et al. 2011; Oliver et al. 2011; Peeters et al. 2013). However, a 24-hour laboratory-based study found this definition over-estimated non-wear time (false-positive misclassification) in overweight and obese adults and adolescents (Choi et al. 2011). The authors recommended using 90-min as opposed to 60-min (Choi et al. 2011). Whether this is applicable to a free-living setting is debatable and other methodological issues with this study have been highlighted (Van Domelen et al. 2011). For example, Choi et al. (2011) only included accelerometer wear-time in their study, they did not assess the effect on the algorithm's ability to correctly classify true non-wear (true-positive rate). Besides sleeping (if following a protocol where the accelerometer is only worn during waking-hours), most activities that require participants to remove their accelerometer are shorter than 90 minutes e.g. swimming, shower, bath etc). Therefore, using a 90 min non-wear time criterion, rather than 60 minute, may increase the false-negative rate (missed detection of true non-wear) more so than reducing the false-positive rate (incorrect classification of true wear as non-wear).

When sleep is part of the investigation, due to its clear association with metabolic health, it is paramount that sleep is not misclassified as non-wear time (Ahmadi et al. 2020). Algorithms have been developed to predict sleep times and again must be carefully chosen to fit the population under investigation. Even better, researchers ought to ensure sound sleep onset and wake time data is gathered, such that sleep time can be defined prior to determining non-wear time.

Researchers need to decide on what constitutes a "valid" day (Migueles et al. 2017). The minimal wear requirement for a valid day is a balance between omitting days when the accelerometer was not worn long enough to accurately represent daily PA, and eliminating too many days, leading to a small sample size and thus less statistical power and/or accurate

representation of daily life. The most common approach is to utilise 10 hours valid daily wear time, as a minimum requirement (Migueles et al. 2017; Tudor-Locke et al. 2012).

Another critical decision to be made when designing an accelerometer protocol is determining the activity cut-point definitions to categorise the time spent in various PA and SB intensities. Cut-points define the count per minute (cpm) threshold for each PA intensity (sedentary, light, moderate, vigorous) (Freedson et al. 1998; Migueles et al. 2017; Troiano et al. 2008; Troiano et al. 2014). They are derived from algorithms most commonly developed using 1) linear regression models, where the line-of-best-fit represents the relationship between accelerometer counts and known energy cost of given lab based activities, or 2) receiver operating characteristics (ROC) curves are used to identify cut-points that provide a good balance between sensitivity and specificity for differentiating PA intensity levels (Jago et al. 2007). The widely used cut-point for SB of <100 cpm was originally based on observation rather than sound scientific evidence (Matthews et al., 2008). However, a study investigating office workers (mean age 47 years) analysed ActiGraph GT3X data and determined that a cut-point of <150 cpm was optimal however <100 cpm was acceptable (Kozey-Keadle et al. 2011). It is important the cut-points used are appropriate for the population under investigation and when comparing studies any differences in cut-points must be acknowledged.

Table 2.4 Methods to measure physical activity and sedentary behaviour (Table adapted from (Fitzhugh 2015; National Institute for Health Research 2022)

Description	Pros	Cons
Subjective		
Questionnaires (interview, self-report) Physical activity questionnaires are completed retrospectively. They vary in their scope and level of detail. Can be validated or not validated.	• Cheap • Low participant burden • Quick	• Varying validity for categorising individuals into groups and ranking activity levels varies greatly between questionnaires • High researcher burden, participant literacy and numeracy required if self-administered • Risk of recall bias • Difficult to recall all SB as it is dispersed throughout the entire day rather than planned bouts that are more memorable to participants (Rääsk et al. 2017)
Diaries and logs Participants record PA in real time/ prospectively.	• Cheap • Allow detailed descriptions of duration, intensity, type and context of PA • Time of activity is recorded, therefore can be time-matched to other data, e.g. accelerometer or heart rate monitor	• High participant burden • Participant requires literacy skills • High level of compliance required therefore unsuitable for younger children (under 10 years) • Participants' may forget to complete diary or log prospectively • Difficult to record all SB as it is dispersed throughout the entire day rather than planned bouts that are more distinct to participants (e.g. swimming, bike ride)(Rääsk et al. 2017)
Direct observation An observer (ideally independent) records PA in real-time.	• Low participant burden • Participant does not require literacy skills • Can obtain detailed information on frequency and duration as well as an estimate of intensity of PA in real time • Accurate information on the type and context of PA e.g. where and with who it occurs	• Expensive and high researcher burden e.g. observation time, data coding • Observers need to be well trained. At risk of reactivity effect (resulting in a change of usual behaviour) due to having an observer present

Description	Pros	Cons
Objective		
Pedometers Device participants wear that measures distance travelled over a given period of time through counting steps.	• Cheap • Low participant burden • Participant does not require literacy skills	• Provide limited information as they do not record PA intensity, frequency or duration and only measure a single plane of movement (up and down) • Only worn for a few days therefore may not capture participation in infrequent activities like social sport participation
Accelerometers Device participants wear that measures acceleration of movement. Tri-axial accelerometers measure movement in three planes; a) vertical (up and down), b) anterior–posterior (side to side) and c) medial-lateral (forwards and backwards).	• Provides valuable, objective, real-time, information on PA and SB patterns including duration, quantity and intensity • Participant does not require literacy skills • Output data can be used to calculate total daily activity PA, which is estimated with an algorithm consisting of a composite of counts from these three planes of motion, named the vector magnitude (VM); $VM = \sqrt{(a^2 + b^2 + c^2)}$.	• Associated data processing software is expensive. Many are not waterproof so cannot capture PA during water based activities e.g. swimming, surfing • Some devices or wear positions are not sensitive to PA in seated or reclining postures, e.g. rowing, cycling or wheelchair use • Not able to account for extra effort required to work against resistance e.g. cycling/running uphill or against wind resistance or lifting weights • Only worn for a few days therefore may not capture participation in infrequent activities like social sport
Heart rate monitors Device participants wear that measures heart rate. An indirect indicator of physical activity as heart rate is a physiological response. Records of heart rate can be used to estimate PA intensity or energy expenditure (EE), using the assumption of a linear relationship between heart rate (HR) and EE (or oxygen consumption, VO_2).	• Easy and quick • Waterproof • Easy to identify non-wear time • Linear relationship between heart rate and EE at moderate-to-vigorous intensity activity	• Relationship between heart rate and EE at light PA and SB is not strong so it will not provide an accurate estimate of activity at these intensities • Indirect measurement of PA – i.e., factors other than PA affect HR e.g. anxiety, fear, excitement, ambient temperature (especially in hot climates), caffeine and some recreational drugs • Only worn for a few days therefore may not capture participation in infrequent activities like social sport
Doubly labelled water method (DLW)	• Reliable and highly accurate at measuring TEE	• Expensive • High subject burden

Description	Pros	Cons
Oxygen-18 (^{18}O) and deuterium (^{2}H) (stable isotopes), are administered as a dose of drinking water (DLW). Timed urine or saliva samples (typically over 7–14 days) are then collected to measure (using isotope ratio mass spectrometry) the elimination rate of these isotopes from the body. The difference between the elimination rates of ^{18}O and ^{2}H in the urine is proportional to CO_2 production, using standard stoichiometric equations this can be converted to average TEE.		• High level of researcher expertise required • No details on frequency, duration, intensity or type of PA performed

EE; Energy expenditure, TEE; Total energy expenditure, PA; Physical activity, SB; Sedentary behaviour, MVPA; moderate-to-vigorous intensity physical activity

2.2.3 Physical activity and sedentary behaviour guidelines

Early PA guidance reflected the research findings that vigorous PA benefits cardiorespiratory fitness (AHA 1972, 1975, ACSM 1978, 1990). As evidence grew, it was recognised that more moderate intensity activities are still beneficial and should not be excluded. A shift in thinking towards a more public health approach to PA subsequently occurred, with the American College of Sports Medicine and the Centres for Disease Control and Prevention establishing PA guidelines for the general public (Pate et al. 1995). Although they are being continually refined, these recommendations still stand today, and have been widely adopted (including in NZ), and include promoting the accumulation of ≥ 30 minutes of moderate or greater intensity activity on ≥ 5 days per week OR 150 min of MVPA per week (Ministry of Health New Zealand 2020). In NZ, a long standing public health message has been "30 minutes a day push play", encouraging New Zealanders to "get moving" (Sport New Zealand 2022). Unfortunately, a 2018 review, including 1.9 million participants and data from across 168 countries, found the global age standardised prevalence of insufficient PA to be 27.5% Guthold, 2018 #411}. Furthermore, women had a higher prevalence compared to men (31.7% and 23.4% respectively) (Guthold et al. 2018). Consequently, physical inactivity, is classified as the fourth leading risk factor for global mortality (World Health Organisation 2017b).

Noticeably absent from these early PA guidelines was any recommendation for light intensity PA or SB. As a result of growing understanding that higher levels of total PA, of any intensity, as well as less time spent in SB are beneficial for health, including reduced risk of premature mortality (Ekelund et al. 2019) (Table 2.5), the PA guidelines in several countries (including Australia, Canada, USA) (Brown et al. 2013; U.S. Department of Health and Human Services 2018) have now been updated. These guidelines not only include promotion of PA in bouts of MVPA but also encourage PA of all intensity and to break up time spent in SB. In 2015 NZ updated their PA guidelines, to include the statements "sit less, move more!" "break up long periods of sitting" and "doing some PA is better than doing none" (Ministry of Health New Zealand 2020). The use of time-use epidemiology in the PA research space is allowing a shift towards 24-hour movement guidelines. Currently Canada promote movement over a 24-hour period, integrating PA, SB and sleep into its movement guidelines (Ross et al. 2020).

In 2021 only 53% of NZ adults reported meeting PA recommendations and along with global trends NZ men were more likely to be considered physically active (57%) when compared to NZ women (49%) (Ministry of Health New Zealand 2021). Further, Pacific and Asian adults

living in NZ were less likely to meet these PA guidelines than non-Pacific (OR; 0.87), non-Asian (OR; 0.80) adults (Ministry of Health New Zealand 2021).

2.2.4 Biomarkers of diseases and health indicators moderated by physical activity and sedentary behaviour

The mechanisms through which PA mediates its beneficial health effects are both numerous and interconnected. Among other effects, increased PA is associated with reduced abdominal adiposity, reduced blood pressure and reduced systemic inflammation, increased insulin sensitivity and glucose homeostasis (Warburton et al. 2006). Further, a reduction in PA often leads to a reduction in total energy expenditure and, if energy intake remains the same, fat mass is gained (Hill et al. 2012).

Physical activity

Moderate to vigorous physical activity is inversely associated with reduced cardiometabolic health, including obesity and T2D (Table 2.5) (da Silva et al. 2019; Gibbs et al. 2017; Knaeps et al. 2016; Nelson et al. 2013; Rosique-Esteban et al. 2017; Swindell et al. 2018). Physical activity improves insulin sensitivity, particularly in overweight and obese weight individuals (Heiston et al. 2020). Even independent of changes to cardiorespiratory fitness, MVPA has been shown to improve insulin resistance in healthy adults (Nelson et al. 2013). However, ordinarily, light PA, along with SB, comprise most of the waking hours. Hence, light PA substantially contributes to total daily energy expenditure. Therefore, when trying to reduce metabolic risk, both MVPA as well as the light intensity PA should be investigated, as excluding light PA from analysis may underestimate the association between PA and metabolic health. In 2017, Fuezeki et al. carried out a systematic review investigating the association of accelerometer-measured light-intensity PA with modifiable health outcomes in adults (≥18 years of age) (Fuezeki et al. 2017). They found that in both the general population and some diseased populations, light PA was positively associated with favourable health outcomes such as reduced obesity prevalence, improved markers of lipid and glucose metabolism, and reduced mortality. Therefore, suggesting inactive individuals should be encouraged to take part in PA of any intensity.

Given the importance of all subcategories of PA intensity, the most important variable to consider may be the total volume of PA (Ekelund et al. 2019). Swindell et. al (2018) (investigated the association among total PA, sedentary time, and cardiometabolic risk (assessed using a range of blood and anthropometric markers) in 2,326 pre-diabetics in a multi-centre (eight country) study. They found, total PA was at least as strongly associated with a

range of cardiometabolic risk markers as MVPA (e.g. triglycerides β=-0.117 and -0.091, $p<0.001$ and CRP β=-0.104 and -0.086, $p<0.001$, for total PA and MVPA respectively). This is also reflected in work by Wolff et. al. (2015), who investigated the association of both minutes of MVPA in bouts (accumulated in > 10 minutes) and total PA with a range of cardio-biomarkers, whilst controlling for a range of confounders including age, sex, ethnicity and BF%. They found significant independent associations between MVPA and triglycerides and insulin. However total PA displayed significant independent associations with eight biomarkers; HDL, triglycerides, plasma glucose, C-peptide, insulin, CRP, homocysteine and systolic blood pressure. Taken together these studies suggest the accumulation of total PA over the day may be as important for metabolic health as achieving the intensity of MVPA in bouts 10 minutes or longer.

Underlying mechanisms by which PA elicits its beneficial effects

Many of the health benefits associated with PA are mediated through its ability to improve glucose and lipid metabolism. The ability for the body to regulate glycaemia and lipids is vital for health; the detrimental effect of poor glucose control (hyperglycaemia) can lead to advanced glycosylated end products (AGEs) and cellular damage whilst poor lipid control can lead to atherosclerosis. Glucose metabolism is controlled by Glucose transporter (GLUTs) transmembrane molecules; they facilitate the transport of glucose out of the extracellular fluid, across the cell membrane and into the muscle or fat cells where it is metabolised. There are several different GLUT molecules across various cell types, however one of these molecules (GLUT4) is dynamic and is translocated to the extracellular membrane upon response to insulin or PA, and is expressed in adipose tissue and striated tissue (cardiac and skeletal tissue). Insulin regulated GLUT4 translocation involves rapid phosphorylation of the insulin receptor, insulin receptor substrate-1/2 on tyrosine residues, and the activation of phosphatidylinositol 3-kinase to initiate translocation of GLUT4 to the cell surface (Folli et al. 1992) (Goodyear et al. 1995). On the other hand, PA has no effect on the insulin receptor, insulin receptor substrate-1/2 tyrosine phosphorylation or on phosphatidylinositol 3-kinase activity (Goodyear et al. 1995; Treadway et al. 1989). Skeletal muscle contraction occurring from PA, activates 5'-AMP-activated protein kinase (AMPK) which is believed to activate translocation of exercise-responsive GLUT4-containing vesicles to the cell surface; this occurs to meet the increased energy demands of skeletal muscle during exercise (Bryant et al. 2002; Shepherd and Kahn 1999). This has been demonstrated in mice without insulin receptors in their skeletal muscle; they maintain normal exercise-stimulated glucose uptake (Wojtaszewski et al. 1999). Taken

together this indicates that insulin and PA mediate GLUT4 translocation in skeletal muscle through distinct signalling mechanisms. This is important in populations with high metabolic disease risk where insulin-stimulated glucose transport may be impaired. Since PA-stimulated glucose transport is not necessary, regularly incorporating PA into the daily routine of at-risk individuals may facilitate the AMPK activation of GLUT4 to utilise glucose for metabolism, thus reducing metabolic disease risk.

Sedentary behaviour

As research continues to confirm the benefits of PA, technological advances encourage the opposite. Since the turn of the millennium, SB has emerged as a distinct health behaviour, independent of PA (Matthews et al. 2012b; Santos et al. 2010). This is due to the relatively weak association between SB and PA (Mansoubi et al. 2014), along with evidence supporting that, even when meeting the guidelines for MVPA, SB is associated with an increased risk for poorer health outcomes (Matthews et al. 2012b).

Hamilton and colleagues have presented compelling evidence, from a mouse model, that chronic bouts of muscular unloading (hindlimb unloading model; rodents hindlimbs were elevated to prevent weightbearing by the hindlimbs, for 10 hours per day for 11 days) resulting from prolonged SB may have harmful biological consequences (Bey and Hamilton 2003; Hamilton et al. 2004). They have suggested the physiological mechanism to be fewer skeletal muscle contractions induced through sitting, consequently resulting in reduced skeletal muscle lipoprotein lipase activity (a key factor for triglyceride uptake and HDL production) and reduced glucose uptake (Bey and Hamilton 2003; Hamilton et al. 2004). Research suggests that not only the quantity of SB, but also the nature in which SB accumulated is detrimental to metabolic health.

Observational studies (Bellettiere et al. 2017; Bellettiere et al. 2019; Diaz et al. 2017), and one experimental study (Saunders et al. 2018) suggested that SB bout duration is associated with biomarkers of metabolic disease risk and all-cause mortality. Diaz and colleagues (2017) investigated associations between SB and mortality risk in a cohort of 7,985 adults (≥45 years old) from the United States of America. After stratifying participants into quartiles according to mean sedentary bout duration, hazard ratios (HR) were calculated to compare quartiles 2 and 4 to quartile 1 for sedentary bout duration. Longer sedentary bout duration was associated with a higher all-cause mortality risk (HR (95% confidence interval): 1.03 (0.67-1.60), 1.22 (0.80-1.85), and 1.96 (1.31-2.93); $p<0.001$). Further, participants classified as

"high" for both total sedentary time (≥12.5 h/day) and sedentary bout length (≥10 min/bout) had the highest risk for all-cause mortality (HR: 2.00 (95% CI: 1.45-2.75); $p<0.001$) (Diaz et al. 2017). In contrast, Jefferis et al. (2019) carried out a prospective cohort study in older British men (N = 7735), one of their aims was to investigate sedentary breaks and bout duration (measured using accelerometers), in relation to mortality. After a median follow-up period of 5 years, they reported no association between breaks in SB or sedentary bout duration and all-cause mortality, in a population of older males (71-92 years). Combined these studies support the *qualitative* public health guidelines on sitting less and breaking up prolonged periods of sitting. However, further evidence is required before clear *quantitative* public health guidance can advise reducing and interrupting sedentary time, to reduce risk for poor metabolic health and death.

2.2.5 Body composition, physical activity and sedentary behaviour

A large amount of cross-sectional, longitudinal and intervention research has been carried out investigating the associations between PA, SB and body composition (Table 2.5). Most research report increased SB is associated with higher BMI and WC, whilst higher MVPA is inversely related to BMI, WC and fat mass (Table 2.5). Some studies have reported that sedentary time has stronger associations with WC (Healy et al. 2008) and other cardiometabolic markers (2 h glucose, triacylglycerol and HDL) (Henson et al. 2013) than either MVPA or total PA. A comprehensive meta-analysis by Ekelund (2016) and colleagues, reviewing 13 studies (including 1,005,791 participants), showed that compared to the reference group (e.g. >35.5 MET-h per week and sitting <4 h/day), those engaging in the least PA (<2.5 MET-h/week and sitting >8 h/day) had the highest mortality rates (HR: 1.27; CI: 1.22-1.31). This study also demonstrated the detrimental effects of SB can be offset by high PA, however, it appears large volumes, between 60 and 70 minutes MVPA per day, are required to do so (Siddique et al. 2017). Considering large review studies (1.9 million participants) have shown just under one third (27.5%) of participants to be meeting the recommendations of at least 30 minutes MVPA per day (Guthold et al. 2018), doubling this recommendation is likely unrealistic for the general population.

A meta-analysis included prospective cohort (23 studies) and one randomised controlled trial aimed to determine the association between SB, body weight and obesity in adults (Campbell et al. 2018). Results showed small, inconsistent and non-significant associations between SB and body weight. For example, whilst SB was associated with an increased risk of becoming overweight or obese (OR 1.33) based on comparing categories of the highest to lowest

sedentary time, it was not meaningfully associated with any measure of body mass e.g. body weight, BMI, or WC. The RCT in Campbell et al.'s (2018) meta-analysis showed no difference in change in BMI between intervention and control groups after 12 months of follow-up (difference 0.41, 95% CI − 1.19 to 2.02; p=0.609) (Biddle et al. 2015). Adding to this Da Silva et al.(da Silva et al. 2019) (2019) aimed to investigate the independent and combined cross-sectional associations of objectively measured PA and sedentary time, as well as longitudinal association of changes in self-reported PA from 23 to 30 years of age with anthropometric and body composition outcomes at 30 years of age. Higher MVPA was associated with lower BMI, WC, visceral abdominal fat, fat mass index and android/gynoid fat ratio. Even after further adjusting for sedentary time, these associations persisted. On the other hand, after controlling for confounding factors (including MVPA), sedentary time was only associated with higher fat mass index (fat mass (kg)/height (m^2), showing that spending increased time in PA appears to improve anthropometric and body composition measurements, however prolonged sedentary time may attenuate the size of the association. Combined, these studies support Hamilton's (2004) aforementioned suggestion that the harmful effects of SB on health, such as diabetes and all-cause mortality, are mediated through distinct mechanisms other than an effect on body composition (Bey and Hamilton 2003; Hamilton et al. 2004).

While these findings have been replicated across diverse populations, very little research has investigated whether PA or SB (time spent in SB and SB's (e.g. bouts and breaks)) are inherently different between high and moderate metabolic disease risk population groups (Table 2.5).

Further, most research has investigated European populations who have a lower prevalence of NCD's when compared to many other ethnic groups (Table 2.5). The threshold level of PA required to confer low metabolic disease risk, may not be the same across all ethnic groups. Indeed, recently researchers have called for future research on SB to focus on overweight/ obese populations as NCD well as ethnic minorities (Katzmarzyk et al. 2019; Schmid et al. 2018).

Table 2.5 Physical activity, sedentary behaviour and metabolic health

Study	Population	Study design	Location	Markers of metabolic health	Measurement of physical activity and cardiorespiratory fitness	Results
(Swindell et al. 2018)	N = 2,326 25–70-year-old Male and female (67%) BMI >25kg.m^{-2} Impaired fasting glucose or glucose tolerance test	Cross-sectional	Multi-centre Denmark, Finland, the Netherlands, Spain, Bulgaria, Australia, New Zealand	Insulin, HbA1c, glucose, CRP, total and LDL cholesterol, HOMA-IR, body composition (DXA, BodPod®)	ActiSleep+ accelerometer, hip mounted, 24-hour protocol for 7 consecutive days	MVPA negatively associated with HOMA-IR ($\beta = -0.122$, $p<0.001$), WC ($\beta = -0.177$, $p<0.001$), fasting insulin ($\beta = -0.115$, $p<0.001$), 2-h glucose ($\beta = -0.069$, $p<0.01$), triglycerides ($\beta = -0.091$, $p<0.001$) CRP ($\beta = -0.086$, $p<0.001$) Total PA (cpm) negatively associated with HOMA-IR ($\beta = -0.151$, $p<0.001$), WC ($\beta = -0.179$, $p<0.001$), fasting insulin ($\beta = -0.139$, $p<0.001$), 2-h glucose ($\beta = -0.088$, $p<0.001$), triglycerides ($\beta = -0.117$, $p<0.001$), CRP ($\beta = -0.104$, $p<0.001$). Sedentary time positively associated with HOMA-IR ($\beta = 0.175$, $p<0.001$), WC ($\beta = 0.215$, $p<0.001$) fasting insulin ($\beta = 0.155$, $p<0.001$), triglycerides ($\beta = 0.106$, $p<0.001$), CRP ($\beta = 0.106$, $p<0.01$), systolic blood pressure ($\beta = 0.078$, $p<0.01$) and diastolic blood pressure ($\beta = 0.057$, $p<0.05$). Analysis was adjusted for the following confounders; age, sex, ethnicity, smoking, household income, education level, BF%, accelerometer wear time, sleep time, and intervention centre. MVPA analysis also adjusted for sedentary time and sedentary time adjusted for MVPA.

Study	Population	Study design	Location	Markers of metabolic health	Measurement of physical activity and cardiorespiratory fitness	Results
(Henson et al. 2013)	N=878 Mean age 63.7 years Male and female (41%) Individuals at high risk of impaired glucose tolerance (questionnaire, BMI, and biochemistry)	Randomised controlled trial	Leicestershire and the South East Midlands region	Fasting plasma glucose and 2 h plasma glucose (via an OGTT), HbA1c, total cholesterol, HDL and triacylglycerol, weight, WC.	Triaxial accelerometer; ActiGraph GT3X, hip mounted, worn during waking hours, for 7 consecutive days	Detrimental linear associations between sedentary time and 2 h plasma glucose (β = 0.220, p< 0.001), triacylglycerol (β = 0.206, p=0.001) and HDL (β = −0.123, p=0.029). Analysis was adjusted for the following confounders; age, sex, smoking status, ethnicity, social deprivation, family history, beta-blockers, lipid-lowering medication, time accelerometer worn, MVPA and BMI. Significant inverse associations between breaks in sedentary time, total physical activity and MVPA and measures of adiposity (WC and or BMI), but not with any other cardiometabolic variables after adjusting for sedentary time and BMI.
(Healy et al. 2008)	N = 169 Male and female (60%) 30-87 years (mean age 53.4 years) Australian Diabetes, Obesity and Lifestyle Study (AusDiab) participants	Cross-sectional	Australia	WC, triglycerides, HDL, resting blood pressure, fasting plasma glucose, and a clustered metabolic risk	Uniaxial accelerometer, ActiGraph model 7164; hip mounted, worn during waking hours for 7 consecutive days	Significant associations of sedentary time, light-intensity time, and mean activity intensity with WC (β=0.22, -0.20, -0.27 respectively) and clustered metabolic risk score (β= 0.23,-0.20, -0.25 respectively). note: p values in table/text presenting data not provided, in text states "significantly associated". MVPA was inversely associated with triglycerides (β= -0.18 , p=0.027), after additionally adjusting for WC. Analysis was adjusted for the following confounders; age, sex, employment status, alcohol intake, household income, education, smoking status, diet quality, family history of diabetes.

Study	Population	Study design	Location	Markers of metabolic health	Measurement of physical activity and cardiorespiratory fitness	Results
Rosique-Esteban 2017 (Rosique-Esteban et al. 2017)	N = 5776 Male (51.9%) and female Mean age 65±4.9y With overweight/obesity BMI ≥27 and <40 kg/m^2, who also met at least three components of the MetS (according to International Diabetes Federation and the American Heart Association and National Heart, Lung and Blood Institute)	Cross-sectional	Spain	BMI ≥30 kg/m^2, WC, systolic and diastolic blood pressure , blood glucose, triglycerides, HDL	Validated Nurses' Health Study questionnaire to assess SB. TV time was used as a proxy for SB's (Martínez-González et al. 2005) Validated REGICOR questionnaire (Molina et al. 2017) to assess leisure-time PA	Increased time in MVPA associated with lower prevalence of obesity, T2D, abdominal obesity and low HDL (Relative Risk (RR): 0.95, 95% CI: 0.93, 0.97; RR: 0.94, 95%CI: 0.89, 0.99; RR: 0.97, 95% CI: 0.96, 0.98; and RR: 0.95, 95% CI: 0.91, 0.99, respectively, all *p*<0.05) Analysis was adjusted for the following confounders; age sex, education level, smoking status, marital status, family history of coronary heart disease, energy-restricted Mediterranean diet adherence. MVPA (when investigating ST), ST (when investigating MVPA). "Each of the other components of the metabolic syndrome" (when assessing associations with each component of the metabolic syndrome).
(Knaeps et al. 2016)	N = 341, Male (61%) and female Mean age 53.8 ± 8.9 years	Cross-sectional	Belgium	A cardio-metabolic risk score, mostly based on the International Diabetes Foundation criteria for metabolic syndrome (International	SenseWear Pro 3 Armband (BodyMedia, Inc, Pittsburgh, PA, USA) 24 hours a day wear protocol, 7 consecutive days	Lower cardiorespiratory fitness (CRF) and MVPA were associated with higher clustered cardio-metabolic risk (β = -0.26 and β = -0.43, respectively, both *p*<0.001). 73% of the variance in the association between MVPA and clustered cardio-metabolic risk was explained by CRF (attenuated this association to non-significance). CRF was the most important risk factor for a higher clustered cardio-metabolic risk after

Study	Population	Study design	Location	Markers of metabolic health	Measurement of physical activity and cardiorespiratory fitness	Results
				Diabetes Foundation 2006)	Cardiorespiratory fitness (maximal exercise test) Peak oxygen uptake (VO_{2peak})	adjustment for MVPA and ST (β = -0.39, *p*<0.001).
(Wolff-Hughes et al. 2015)	N = 8228 Mean age 46.5 years Male and female (50.6%) 74.3% Non-Hispanic Whites Data from 2003-2004 and 2005-2006 NHANES cycles.	Cross-sectional	United States of America	Systolic blood pressure, diastolic blood pressure, BMI, WC, triceps skinfolds, and subscapular skinfolds, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, glycohemoglobin, plasma glucose, C-peptide, insulin, C-reactive protein, and homocysteine.	ActiGraph model 7164 activity monitor, hip mounted, worn during waking hours for 7 days	Adj. Wald F statistics HDL - (MVPA = 0.49 *p*>0.05 VS total activity counts (TAC) = 14.08, *p*<0.01) Plasma glucose (MVPA = 1.08 *p*>0.05 vs TAC = 13.92, *p*<0.001) C-peptide (MVPA = 0.04 *p*>0.05 vs TAC = 29.10, *p*<0.0001) Insulin (MVPA = 0.24 *p*>0.05 vs TAC = 19.74 *p*<0.0001) CRP (MVPA = 1.29, *p*>0.05 vs TAC = 26.67, *p*<0.0001) Homocysteine (MVPA = 0.57, *p*>0.05 vs TAC = 7.71, *p*>0.01) systolic blood pressure (MVPA = 0.001, *p*>0.05 vs TAC = 4.43, *p*<0.05) BMI (MVPA= 8.72, *p*=0-.01, *p*<0.01, TAC = 6.91, *p*<0.05) WC (MVPA = 2.49, *p*>0.05, TAC = 20.28, *p*<0.001) Triceps skinfold (MVPA = 0.28, *p*<0.05, TAC = 14.97, *p*<0.001) Subscapular skinfold (MVPA = 0.99, *p*>0.05 vs TAC = 5.62, *p*<0.05)

Study	Population	Study design	Location	Markers of metabolic health	Measurement of physical activity and cardiorespiratory fitness	Results
						Triglycerides (MVPA = 8.12, $p<0.01$ vs TAC = 4.55 $p<0.05$) Analysis was adjusted for the following confounders; age, gender, race, education, smoking, general perceived health, , osteoporosis, coronary heart disease, angina, heart attack. Also BMI, diabetes, high blood pressure when appropriate.
(Nelson et al. 2013)	N=402 Male and female (47%) Mean age 32.6 years NHANES data, healthy individuals	Cross-sectional	United States of America	Fasting glucose and insulin. HOMA-IR, DXA	Actigraph AM-7164 accelerometer, hip mounted, during waking hours for 7 consecutive days VO_{max}	Strong significant associations between MVPA and log HOMA-IR (β= −0.1607, p=0.004). No significant association between cardiorespiratory fitness and log HOMA-IR. Analysis was adjusted for the following confounders; body fat %, sex, age, cardiovascular fitness.
(Gibbs et al. 2017)	N=1826 Age 38-50 years Male and female (57%) Participants from the coronary artery and risk development in young adults longitudinal cohort study	Longitudinal follow up at 5 years	United States of America	BMI, WC CARDIA diet history	Only measured at baseline – ActiGraph uniaxial accelerometer (model 7164), worn during waking hours for 7 consecutive days	Baseline: Total and prolonged ST was directly associated with BMI and WC whereas MVPA was inversely related (all $p<0.05$). Longitudinally: Only prolonged ST was associated with higher BMI (0.077, p=0.033) and WC (0.198 cm, p=0.028). Analysis was adjusted for the following confounders; age, race, gender, centre, education, alcohol intake, smoking status, energy intake wear time and baseline value (for 5 year changes models), MVPA (when investigating ST), ST (when investigating MVPA).
(da Silva et al. 2019)	N = 3206	Cross-sectional and	Brazil	DXA, WC, BMI, fat mass index	GENEActiv accelerometer,	Cross-sectional: Higher MVPA associated with lower BMI (β = -0.017), WC (β = −0.043) visceral

Study	Population	Study design	Location	Markers of metabolic health	Measurement of physical activity and cardiorespiratory fitness	Results
	Male (49.6%) and female Baseline 23 years old, follow up 30 years old	longitudinal (follow up at 7 years)		(Fat mass (kg)/height (m^2)	triaxial, wrist mounted, (only measured at 30 years), 24 hours a day wear protocol, 4-7 consecutive days 26 years: International physical activity questionnaire 30 years: "a self-reported questionnaire regarding the duration and weekly frequency of leisure-time PA"	abdominal fat (β = −0.006), and fat mass index (β = −0.015). Sedentary time independently associated with higher fat mass index (β = 0.003); p values not reported. Longitudinal analyses (questionnaire): adiposity was lower among participants who were consistently active or who became active. Adiposity was comparable between the "became inactive" and "consistently inactive" participants. Analysis was adjusted for the following confounders; gender, family income at birth, maternal schooling at birth, maternal skin colour, birth weight, socioeconomic status and achieved schooling of the member cohort at 23 years, smoking at 23 years, daily energy intake at 23 years. BMI and WC, sedentary time and MVPA when appropriate.

BF%; body fat percent, BodPod®; an Air Displacement Plethysmography system, DXA; Dual-energy x-ray absorptiometry, cpm; accelerometer counts per minute, WC; waist circumference, LDL; low density lipoprotein, HDL; high density lipoprotein, CRP; C-reactive protein; HOMA-IR; Homeostatic Model Assessment for Insulin Resistance, HbA1c; blood glycated haemoglobin, ST; sedentary time, SB; sedentary behaviour.

2.3 Diet

Although the time devoted to PA and SB influences energy expenditure, diet directly affects energy intake. Dietary intake is in part driven by a web of endocrine regulators, stimulating hunger (e.g. increased glucagon and ghrelin) and satiety (increased blood glucose, insulin and leptin). As previously discussed, changes to these endocrine regulators are common in an obese state (Xiao et al. 2020). Globally it appears diets are becoming more "westernised" (Imamura et al. 2015). A systematic review of diet among 187 countries revealed that based on their dietary pattern scores, global consumption of "healthy" items improved between 1990 and 2010, whilst consumption of "unhealthy" foods, high in saturated fat, salt and sugar worsened (Imamura et al. 2015). Further, there was heterogeneity across regions and countries, highlighting the importance of region and country specific cultural differences when investigating diet.

2.3.1 Measurement of diet

Finding the balance between selecting a reliable, valid dietary assessment method, that is also feasible from a cost and participant burden perspective is challenging (Gibson 2005). Several methods have been developed to estimate dietary intake, these can be broadly classed as prospective (food diaries) or retrospective (food frequency questionnaire, diet history, diet recall) and each have advantages and disadvantages (Table 2.6).

The validity of dietary data can be compromised due to incorrect dietary assessment tool selection or measurement errors. This includes systematic errors, such as under and over reporting, limitation of dietary data bases for data processing, and random errors like erroneous data recording or entry (Gibson 2005). All these errors can compromise the interpretation of the relationship between diet, obesity and other NCD's. Importantly, doubly labelled water studies have highlighted how pervasive under reporting of dietary intake is with self-report dietary assessment methods (Gibson 2005). Under reporting is when participants, whether it be individuals or populations, subconsciously or consciously (social desirability bias), report dietary energy intakes that are below their true intake, and in some instances not biologically viable (Gibson 2005). Methods such as the Goldberg cut off method have been developed to identify under and over reporting. This method assumes participants are in energy balance and is calculated as the ratio of the mean daily reported energy intake to estimated basal metabolic rate. This ratio is then compared to predetermined cut offs that represent the value below which it is statistically it is unlikely the reported ratio of energy intake to BMR would realistically reflect habitual daily energy intake of a person with a sedentary lifestyle (1.55 x basal metabolic

rate) (Gibson 2005). Others have suggested epidemiological cut offs including >500kcal/day and <3500kcal/day for women and >800kcal/day and <4000kcal/day for men (Willett 2012). Higher cut-offs have also been proposed for research in ethnic minority groups (George et al. 2004; Kolonel et al. 2000). Factors unique to the population under investigation need to be considered, such as PA levels and energy expenditure when exploring the plausibility of the dietary data, before excluding participants based on cut-offs (Cade et al. 2017). Once identified, under and over reporters are usually excluded from data analysis. It is known that women are more likely to underreport when compared to men and under reporting is also more prevalent among obese or overweight individuals when compared to lean subjects (Archundia Herrera and Chan 2018). Unfortunately, currently no self-report dietary intake method is immune to under reporting thus data analysis needs to consider this.

Table 2.6 Comparison of Methods to measure dietary intake (Table adapted from (Gibson 2005) and (Willett 2012))

Assessment tool	Description	Advantages	Disadvantages
	Prospective		
Weighed food record	The most precise method for estimating the amount of food and nutrients usually consumed (Gibson 2005). Participants (or support persons) record, weight (using kitchen scales) and details of all foods and beverages as they are consumed, over a given time period (typically one to seven days). Often two-dimensional representations of different shapes (such as a match box) and photographs participants have taken of foods/beverages are used to help the researcher convert volume or area descriptions to estimates of mass (g or kg).	Provides detailed information on timing of meals, portion size (objective actual amount consumed), and description of the food without reliance on memory. Higher reproducibility compared to estimated food record. Can capture differences between weekday and weekend intake.	High participant burden. Requires sound literacy and numeracy skills and high degree of co-operation from participant. Moderate researcher burden due to the labour intensive task of inputting data into data analysis software. Ideally requires skilled researchers and the use of coding rules. Important to ensure even representation of all days of the week within the population.
Estimated food record	Same as weighed food record except instead of kitchen scales amount consumed is estimated (subjective)- Often household measures are used (jugs, cups, bowls, spoons) which have been supplied and/or previously calibrated by researchers, or by means of a set of standard measures. Often two-dimensional representations of different shapes (such as a match box) and photographs participants have taken of foods/beverages are used to help the researcher convert volume or area descriptions to estimates of mass (g or kg).	Provides detailed information on timing of meals, portion size (subjective estimate of amount consumed), and description of the food without reliance on memory Can capture differences between weekday and weekend intake Less disruption to participant normal eating patterns and food habits than the weighing of food.	Moderate participant burden. Requires sound literacy skills. Moderate researcher burden due to the labour intensive task of inputting data into data analysis software. Ideally requires skilled researchers and the use of coding rules. Relies on participants ability to describe portion sizes. Important to ensure even representation of all days of the week within the population.
	Retrospective		

Assessment tool	Description	Advantages	Disadvantages
24-hour recall	Structured interview, intended to capture detailed information about all foods and beverages (types and portions) consumed by the participant in the past 24 hours. Often used for estimating large populations dietary intake (e.g. national nutrition surveys). Often two-dimensional representations of different shapes (such as a match box) and photographs participants have taken of foods/beverages are used to help the researcher convert volume or area descriptions to estimates of mass (g or kg).	Quick and low cost. Minimal participant burden. Moderate researcher burden. Can be completed in person or via remote access (phone/online etc.)	Relies on memory. Requires a skilled, trained interviewer. Participant is estimating portions in retrospect.
Food frequency questionnaire	Assess usual dietary consumption over a given time period (e.g. weekly, monthly or yearly), through questioning the usual frequency participants consume food and beverages from a finite, pre-defined food list. Frequency categories range from never or less than once per week/month/year up to ≥6 times per day and participants have to select one of these options. Depending on the FFQ design, the length of the list of foods can range from about 20 to 200 items. Reported intake is limited to the foods contained in the food list. Depending on the range of foods and beverages queried, data can be used to assess total dietary intake and or certain aspects of diet e.g. specific nutrients. Quantitative and semi-quantitative FFQs include portion sizes to obtain quantitative data.	Low to moderate participant burden, depending on length. Inexpensive to administer and process in large populations, especially when it is done online or via a machine readable answer sheet. Participant can complete independently. Low to moderate researcher and participant burden. Does not influence dietary behaviour. Does not require well trained interviewers.	Relies on memory. Requires literacy and numeracy skills. Memory of past diet may be influenced by present diet. Complex therefore not appropriate for children. Requires skilled researcher to design FFQ tool, especially when validated.
Diet history	Trained interviewer questions participants to assess and estimate usual dietary intake and meal patterns over a pre-determined period (usually 1 month).	Low participant burden. Estimates usual dietary intake and meal pattern over a long period of time.	Relies on memory. Requires a skilled, trained interviewer. High researcher burden

Assessment tool	Description	Advantages	Disadvantages
		Can take seasonal variation of diet into account. Does not influence dietary behaviour. Can classify participants into food consumption categories.	Participant is estimating portions in retrospect. Mis-reporting is common. Compared to diet records dietary intake more likely to be overestimated. Some foods may not be reported due to social desirability.

2.3.2 Dietary guidelines

Whilst the concept of a healthy diet is not new, what constitutes a healthy diet is constantly being refined. Current global and NZ dietary guidelines encourage an increased intake of fruits and vegetables, whole grain cereals and fibre and reduced saturated fat, free sugars, refined starch, and sodium (Ministry of Health New Zealand 2020; World Health Organisation 2020b). Also in 2019, in attempt to guide a global transformation of the food system, the EAT–*Lancet* report provided a road map of an optimal dietary pattern to promote both human health as well as the environmental health of the planet (Willett et al. 2019). The recommended diet includes a high intake of plant-based foods including vegetables, fruits, whole grains, legumes, nuts, and unsaturated oils, a low to moderate amount of seafood and poultry, and no or very little red meat, processed meat, added sugar, refined grains, and starchy vegetables.

2.3.3 Diet and metabolic health

The Global Burden of Disease Study investigated the associations between food consumption and NCD's and mortality across 195 countries (Afshin et al. 2019). They reported that insufficient dietary intake including core 'healthy' foods and nutrients (e.g., fruit, vegetables, wholegrains, and dietary fibre), and excessive intake of non-essential 'discretionary' foods and nutrients (e.g., sugar sweetened beverages, red and processed meats and sodium) was the leading cause of death compared to all other risk factors for NCDs, including tobacco smoking. A Western style diet, typified by high intakes of discretionary foods including; refined grains, processed meat, fried foods, red meat and low in fibre has been negatively associated with higher body weight, BMI, adiposity and waist to hip ratio and increased risk of NCDs' including T2D (Bell et al. 2015; Ludwig et al. 2018; Paradis et al. 2009; Singh et al. 2017). Conversely, a "prudent" or "healthy" dietary pattern (fruits, vegetables, wholegrains, eggs, fish and seafood), is persistently associated with a healthier body composition (Beck et al. 2018; Newby et al. 2003; Paradis et al. 2009; Suliga et al. 2015), metabolic regulation, favoured lipid profile and reduced inflammation (Esposito et al. 2013; Kastorini et al. 2011).

Specific to the NZ setting, Jayasinghe et al. (2019) carried out a comprehensive investigation in a cohort of premenopausal Pacific, Māori and European, NZ women, looking at the relationship between dietary patterns, body composition and metabolic biomarkers. Findings showed women with higher scores for a 'refined and processed' dietary pattern had higher BMI and adiposity, higher circulating levels of insulin and leptin, and lower levels of ghrelin compared to those with lower scores. The 'refined and processed' dietary pattern was dominated by discretionary foods, including deep fried and fast food, refined grains and soft drinks. When stratified by ethnicity, they also found more Māori (51%) and Pacific (68%)

women and fewer NZE (16%) women followed the ‘refined and processed’ pattern, whereas more NZE women followed the ‘sweet and savoury snacking’ pattern. A greater understanding of the dietary practices of NZ Māori and Pacific women compared to NZE women may contribute towards understanding and addressing obesity and metabolic disease risks in these populations.

Researchers agree that dietary, in combination with PA interventions, are more effective than attempting to modify these behaviours independently (Alamian and Paradis 2012; Alvarez-Alvarez et al. 2018; Elliot and Hamlin 2018). PA induces changes in hormones implicated in appetite regulation (including leptin, ghrelin, peptide YY, glucagon-like peptide-1 and insulin) (Gondim et al. 2015; Schubert et al. 2014). Therefore being habitually physically active allows individuals to have a heightened sensitivity to their appetite regulation, better matching energy intake with energy expenditure (Blundell et al. 2003; Dorling et al. 2018; Hopkins and Blundell 2016).

2.3.4 Socioeconomic status and diet

Higher socioeconomic status is consistently associated with consuming a higher quality diet (Darmon and Drewnowski 2008), including higher intake of fibre, vitamin A and C, folate, calcium and iron (Larson and Story 2009). In contrast, people who live in deprived communities have reduced food security, for example poorer access to fruit and vegetables (Cummins and Macintyre 2006; Eagle et al. 2012), which could lead to a poorer diet quality. Lower quality diets (comprised of high sugar and fat content) as well as energy dense foods (comprised of refined grains, added sugars, or fats) have been found to be cheaper on a per-kilojoule basis and lower income groups tend to select cheaper and more energy dense diets (Darmon and Drewnowski 2015). Living in the most deprived areas in NZ decreases the likelihood of consuming the recommended daily servings of fruit and vegetables by 33%, in comparison to individuals living in the least deprived areas (Ministry of Health New Zealand 2021). The 2008/09 NZ National Nutrition Survey found that Pacific women were nearly three times more likely to live in a household that had low food security compared to non-Pacific women (Ministry of Health New Zealand 2012).

These differences in diet quality between high and low socioeconomic strata can be explained by a number of environmental factors including, differences in availability of healthy foods between these neighbourhoods, lack of time and/or resources for food preparation and economic barriers to purchase healthy food (Larson and Story 2009).

2.4 Gut microbiota

The gut microbiota is a complex ecological community of commensal, symbiotic and pathogenic micro-organisms. The gut microbiota plays a pivotal role in nutrient metabolism, as there are several types of carbohydrates, including dietary fibres, such as resistant starch, cellulose, xylans and inulin, that human enzymes cannot digest (Chambers et al. 2018). When these carbohydrates are fermented and metabolised by the gut microbiota, the primary products of their breakdown are short chain fatty acids (SCFAs), such as acetate, propionate and butyrate (Liu et al. 2022). These SCFA's play a significant role in gut and general health, including acting as an energy source for microbes, modulating inflammation, and gut motility (Liu et al. 2022). SCFA's also assist with regulating satiety, therefore helping to regulate food intake (Liu et al. 2022). In animal models of obesity, butyrate has been shown to increase energy expenditure, improve insulin sensitivity, and reduce adiposity (Gao et al. 2009). Thus an imbalance in healthy and unhealthy microbes in the gut (dysbiosis) may contribute to host health, including obesity and T2D.

2.4.1 Measurement of gut microbiota

Since the early 2000's high throughput, microbiota sequencing techniques, have greatly evolved and are now much more accessible for research. The most cost effective, commonly used sequence technique is 16 rRNA gene sequencing (Goodrich et al. 2014), however shotgun metagenomics is being used more frequently, as it has become more affordable. While 16s rRNA only reads one region of the DNA (16s rRNA genes), shotgun metagenomics can read all genomic DNA in a sample. Shotgun metagenomics can characterise down to the species level (or sometimes strain level), however the taxonomic resolution is more limited with 16s rRNA, with characterisation typically down to the family or genus level (Allaband et al. 2019). 16s rRNA is also more prone to sequencing errors and differences as a result of different regions chosen for analysis (e.g., hypervariable region V3 vs V4) (Janda and Abbott 2007).

The gut microbiota is made up of various bacterial species, taxonomically classified by genus, family, order and phyla. Increased access to the aforementioned sequencing techniques has allowed microbiologists to identify with confidence, the main bacterial phyla within the human faeces; *Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria and Verrucomicrobia*. The two most prominent phyla being *Firmicutes* and *Bacteroidetes* (Tannock 2017). Examples of families within the *Firmicutes* phyla are *Ruminococcaceae, Eubacteriaceae and Lachnospiraceae* and examples of families within the *Bacteroidetes* phyla are *Rikenellaceae*, *Prevotellaceae* and *Bacteroidaceae* (Tannock 2017). Individual humans' gut microbiota are shaped in infancy, and influenced by a number of factors including, mode

of delivery at birth, method of infant feeding, use of certain medications (e.g. antibiotics) and genetics (Wen and Duffy 2017). This “native” gut microbiota remains relatively stable throughout life, however is influenced by physical activity, cultural and dietary habits, body composition and age (Lozupone et al. 2012). Thus a reference composition of a “healthy” gut microbiota has not been defined and is different for everyone. However, a richer more diverse microbiota does characterise a more healthy gut microbiota (Fan and Pedersen 2021), and diversion from this or “dysbiosis” characterises a less health gut microbiota (Rinninella et al. 2019). A high fibre diet such as wholegrains, vegetables, fruit and legumes is known to increase the microbial diversity.

Microbial dysbiosis can be determined using a range of measures, including but not limited to, diversity (α or β), species richness or microbial abundances such as relative abundance (%) at various taxonomic levels (e.g. family, genera or species) and alterations in the ratio between the dominant phyla Firmicutes and Bacteroidetes (*Firmicutes:Bacteroidetes*) (Magne et al. 2020). Decreased microbiota diversity and richness and a higher *Firmicutes:Bacteroidetes* ratio (although no ideal ratio has been formally defined) have been associated with obesity, T2D and altered blood glucose (Ley et al. 2006). However the abundance of *Firmicutes* in the gut microbiota of healthy individuals been shown to vary between 11% to 95% and that of Bacteroidetes between 0.6% to 86.6%, therefore an optimal *Firmicutes:Bacteroidetes* ratio for human health has not been defined (Magne et al. 2020). Alpha (α) diversity, is the average species diversity (similarity or dissimilarity) within a single sample while measures of beta (β) diversity, indicate diversity between populations (Qian et al. 2020). There are a number of different metrics commonly used to quantify α diversity, these can be broken down into three general categories: 1) measures of species richness, 2) measures of species evenness, 3) measures that consider richness and evenness. Species richness is the number of species at a specific site (species count, operational taxonomic units (OTU’s)) (Qian et al. 2020). Species evenness indicates how evenly distributed abundances are between all present species at a site (e.g. Pielou’s Evenness) and overall diversity metrics such as Shannon index consider both richness and evenness. Many metrics also exist to measure β diversity, such as Unifrac distance or Bray-Curtis (Qian et al. 2020). They all consider different aspects of community heterogeneity, including shape (abundance of each taxon) and size (overall abundance per sample) . Bray-Curtis is a common example of a metric that considers shape as well as size.

2.4.2 Link between gut microbiota and obesity

The seminal work of Jeffrey Gordons and colleges demonstrated causality between the gut microbiota, obesity and metabolic abnormalities (Bäckhed et al. 2004). Lean germ-free mice were infected with faecal matter from conventionally raised mice (i.e., mice raised in contact with microbes) and whilst there was no increase in calorie intake, the mice increased in weight by 60% and developed insulin resistance after 14 days (Bäckhed et al. 2004). Adding to this, germ-free mice received faecal matter from either lean or obese mice whilst calorie intake was controlled. (Turnbaugh et al. 2006) After two weeks, it was noted that the mice who received faecal matter from the obese donors became obese, and those who received lean faecal matter remained lean (Turnbaugh et al. 2006). In a similar study, lean, germ-free mice were fed faeces from either an obese or lean human twin. Calorie intake was controlled and the mice fed obese faeces become overweight, and the mice fed lean faeces remained lean; when the mice were housed together and ate each other's faeces, their weights became similar (Ridaura et al. 2013). These findings provide plausible evidence of a relationship between the gut microbiota and obesity.

Human studies are more limited, although obese individuals do appear to show a difference in their gut microbiota composition when compared to their leaner counterparts (Bouter et al. 2017; Turnbaugh et al. 2009) (Ley 2010; Ravussin et al. 2012). It was initially speculated that, a healthy, lean human had a higher *Firmicutes:Bacteroidetes* ratio, and higher diversity and richness of the microbiota when compared to individuals with obesity (Ley et al. 2006; Turnbaugh et al. 2006). When lean participants and participants with obesity followed a lower calorie diet for 52 weeks, the *Firmicutes:Bacteroidetes* ratio of participants with obesity approached the same proportions as observed in the lean group (Ley et al. 2006; Turnbaugh et al. 2006). However a recent review concluded that the evidence associating the proportions of bacterial phyla *Firmicutes* and *Bacteroidetes* with obesity, "is not convincing" and is still a matter of debate (Magne et al. 2020). Methodological differences in studies, poor characterisation of populations under investigation and failure to consider lifestyle factors such as diet, PA and antibiotic use, known to affect gut microbiota composition and diversity appear to confound the observed effect (Magne et al. 2020).

2.4.3 Physical activity and the gut microbiota

For some individuals, PA at the extreme end of the spectrum (intense prolonged PA), such as marathon running, negatively affects the digestive system, causing "exercise-induced gastrointestinal syndrome" with symptoms, such as nausea, vomiting, abdominal pain, flatulence, or diarrhoea (Ribeiro et al. 2021). However, moderate levels of PA tend to have a

beneficial effect on absorption of nutrients including electrolytes, intestinal permeability, as well as the rate of excretion of toxic metabolic products (Peters et al. 1999). It is thought these gastrointestinal symptoms may be associated with PA induced alterations to the gut microbiota makeup, including taxonomic and community changes, although knowledge on the underlying mechanism is poorly understood (Mailing et al. 2019) (Table 2.7).

Early animal research demonstrated that, in rats, 12 weeks of increased voluntary running exercise, resulted in increased microbiota diversity (Shannon diversity index, ($F(1,20 = 7.8$, $p=0.001$)) (Evans et al. 2014). For mice fed high fat diets (to induce obesity), exercise protected them from obesity and there were associated changes in the ratio of the four major phyla (*Bacteroidetes, Actinobacteria, Proteobacteria and Firmicutes*) of the gut microbiota. The changes associated with exercise or a high fat diet alone, were in the same direction, although the magnitude in bacterial diversity was greater in the combined high fat and exercise group. An inverse relationship between total distance run and the *Firmicutes:Bacteroidetes* ratio was also reported. However, this relationship may not be clear cut, as findings by Kang et al. (Kang et al. 2014), where the relationship between exercise and the gut microbiota in mice was determined to be independent. It is important to note that in animal studies the metabolic health of the animal may play a role in determining PA-induced changes in the microbiota. Studies involving diabetic mice have reported different microbiota shifts compared to non-diabetic mice after six weeks of exercise (Lambert et al. 2015).

The first study to explore changes in the gut microbiota of healthy human individuals in relation to PA found that compared to size-matched controls, professional male rugby player's gut microbiota had a greater diversity within the *Firmicutes* phyla (Clarke et al. 2014a) (Table 2.7). It was proposed that greater diversity within the *Firmicutes* phyla may help maintain a healthier intestinal environment. However, because the diet of the athletes differed significantly from the diet of the controls; especially higher protein intakes, the specific role of PA could not be conclusively established (Clarke et al. 2014b). The faecal samples of the participants were later re-analysed using metagenome shotgun sequencing allowing more specific detail on taxonomic composition and functional potential of the microbiota (Barton et al. 2018). Results revealed that more prominent differences in the microbiota existed at the functional metabolic level rather than the compositional level when comparing athlete to controls. Specifically looking at SCFAs, higher levels of acetate, propionate, butyrate and valerate were observed in athletes compared to controls. However, as with the initial study, whilst there was a significant difference between elite male athletes and sedentary individuals, due to the potential

confounding factor of diet, the association with PA could not be determined (Barton et al. 2018).

Case studies and prospective studies in well-trained endurance athletes seem to reveal clear results. Four well-trained male athletes provided stool samples for metagenomic shotgun sequencing before, during and after a continuous, 33-day, 5000 km transoceanic rowing race (Keohane et al. 2019). Results revealed that microbial diversity increased throughout the ultra-endurance event. This included an increased amount of butyrate producing species and *Dorea longicatena* which has been positively associated with insulin sensitivity (Brahe et al. 2015). Interestingly at the three month follow up many of the adaptions in microbial community structure persisted (Keohane et al. 2019). However increased microbiota alpha diversity isn't always a positive indicator for gut health, as it may be associated with the growth of potentially harmful bacteria such as Proteobacteria (Karl et al. 2017). Also, a "world class" marathon runner demonstrated an increase in alpha diversity, two hours post-race (Grosicki et al. 2019). However when investigating the specific taxonomic changes of the marathon runner, some of the increases were in the potentially harmful *Haemophilus* and *Streptococcus* genera. These findings provide supporting evidence that acute, endurance PA likely contributes to alterations in human gut microbiota, however these changes are not always beneficial.

Others have assessed long term PA volume and its association with the gut microbiota (Hampton-Marcell et al. 2020) (Table 2.7). To capture a reduction in training volume through the season, division one collegiate swimmers were assessed during peak training through their in-season taper (Hampton-Marcell et al. 2020). Although no significant changes in body composition were found, a significant decrease in microbial α-diversity as well as a decrease in the proportion of the butyrate producing bacteria *Faecalibacterium* (phylum *Firmicutes*) and *Coprococcus* (phylum *Bacillota*) was observed. It is important to note that dietary data was not collected and therefore not controlled for in this study. Also, in a cohort of cyclists, after controlling for dietary intake and independent of being professional or amateur, frequency of training was positively associated with counts of faecal *Prevotella* (phylum *Bacteroidetes*), a fast growing bacteria involved in energy and carbohydrate metabolism (Petersen et al. 2017). This study however used "questionnaires on diet" and "alcohol consumption", thus no in-depth dietary data was collected (Table 2.7). Combined these studies suggest that among humans the gut microbiota appears to be altered, following changes in PA volume, however the aforementioned associations may, at least in part, be a result of differences in diet, including carbohydrate intake rather than PA.

The gut microbiota of individuals with varying levels of cardiorespiratory fitness as measured by peak oxygen uptake (VO2 peak) has been investigated (Estaki et al. 2016). Using a dietary recall method, participants' diets were matched and it was demonstrated that cardiorespiratory fitness was positively correlated with increased gut microbial alpha diversity and a higher production of the SCFA, butyrate. This finding supports the thought that increased physical fitness may support overall gut homeostasis and health through changes in the gut microbiota. They also reported a positive association between cardiorespiratory fitness and faecal counts for a number of bacteria from the phylum *Firmicutes* (specifically; order *Clostridiales* family *Lachnospiraceae*, family *Erysipelotrichaceae*, genus *Coprococcus*, genus *Roseburia* and genus *Adlercreutzia* (phylum *Actinobacteria*). More recently, investigating healthy young adults, a significant (significance set at $p>0.004$), positive association between *Firmicutes:Bacteroidetes* ratio and cardiorespiratory fitness ($r = 0.48$, $p<0.003$) was reported, but not with other fitness, dietary intake, or anthropometric variables ($p>0.004$) (Durk et al. 2019).

It is important to note that all of the aforementioned studies have included elite or recreational athletes, therefore, associations between PA and the gut microbiota in the general public are less clear. Investigating community-dwelling older men, Langsetmo et al. (2019) reported no consistent pattern by phylum and objectively measured PA was not associated with α-diversity. However, they reported a weak statistically significant association between self-reported PA and β-diversity after adjustment for covariates, suggesting that PA only has a small effect on variation among core taxa. Adding to this, when investigating the gut microbiota of sedentary and physically active (WHO guidelines) premenopausal women, it was reported that after adjusting for dietary intake, PA was not associated with differences in microbiota diversity or richness (both the total number of OTUs and species recorded) (Bressa et al. 2017). This study did however find a negative association between SB and microbiota richness as well as a positive association between PA and faecal counts for genus *Bifidobacterium* (phylum *Actinobacteria*) and *Akkermansia muciniphila* (phylum *Verrucomicrobia*). *Akkermansia muciniphila* has been associated with improved metabolic markers, the stimulation of gut peptide hormone secretion, improved gut integrity, and the reduction of metabolic inflammation (Si et al. 2022). Also, *Bifidobacterium* is a lactic acid-producing bacteria and due to their positive association with health are commonly used in probiotics (Sharma et al. 2021). In contrast to Bressa et al. (2017), among highly trained athletes, Barton et al. (2018) reported a reduction in *Bifidobacterium* abundance with

increased PA. Taken together these observational studies support the thought that PA is associated with positive alterations to the human gut microbiota diversity and taxonomic composition. It appears these associations are strongest in trained, physically fit individuals, but are only beneficial to a certain PA threshold, at which it then may have a negative impact on the gut microbiota. It is not clear what level of PA is needed to induce these positive changes to the gut microbiota. Further, the confounding factors of diet and possibly sleep are an issue, and prevent any clear conclusion from being drawn, therefore these factors must be considered in future research.

This is a ripe area of research but the heterogeneity between study methodology precludes any firm conclusions from being drawn. For example, variations in PA type and duration, how physically fit participants are or the metabolic state of participants, however it is difficult to isolate the effect of each individual variable. The cohorts of participants included in this area of research are extremely diverse. Most human studies investigate elite athletes or physically fit individuals. Who are more physically fit and tend to have a unique dietary profile when compared to non-athletic individuals who pursue recreational PA (Clarke et al. 2014a). The majority of the general population is untrained, therefore to improve the reliability of any associations seen between PA and the gut microbiota in the general public, especially high metabolic disease risk populations, more studies are needed. In addition to this there has been a call for research to focus on the contribution of current WHO PA dose recommendations on gut microbiota composition (Shahar et al. 2020). This will help further guide PA recommendations for the general population. Most research has been carried out in European populations (Table 2.7). Despite the knowledge of variations in the gut microbiota composition of healthy individuals from different ethnic groups (Deschasaux et al. 2018), very few have investigated ethnic minorities' associations between PA and the gut microbiota (Table 2.7). Thus it is important that minority ethnic groups are included in future research as their unique gut microbiota profile may respond differently to PA when compared to European populations.

Further, within this area of research, methods of measuring PA are inconsistent. Most studies use self-reported questionnaires to determine PA (Barton et al. 2018; Clarke et al. 2014b; Mörkl et al. 2017; Petersen et al. 2017), only some of these being validated (Barton et al. 2018; Clarke et al. 2014b; Mörkl et al. 2017). Very few used objective measures of PA such as accelerometers (Langsetmo et al. 2019) (Bressa et al. 2017).

The intimate relationship between PA and diet is also evident in research on the gut microbiota. This indicates that when the true or specific relationship between PA and the

composition and activity of the gut microbiota is determined, the confounding influence of dietary intake must be considered to deem any findings relevant; it is paramount that diet is controlled for in any statistical modelling (Turnbaugh et al. 2006; Q. Yang et al. 2020). Although many studies gather dietary intake information they fail to control for its confounding effect (Barton et al. 2018; Clarke et al. 2014b; Durk et al. 2019; Estaki et al. 2016; Mörkl et al. 2017). Further, growing evidence is highlighting the impact that sleep behaviour has on the gut microbiota, a confounder none of the aforementioned studies controlled for and future studies should seriously consider (Thaiss et al. 2014). Lastly, gut microbiota studies do not always control for other important confounding variables including gender, ethnicity, gastrointestinal issues and the use of antibiotics.

Table 2.7 Physical activity and the gut microbiota

Study	Participants	Study design	Microbiota analysis	PA and cardiorespiratory fitness assessment	Co-variates	Results
(Clarke et al. 2014a) Ireland	N=86 Professional male rugby athletes (n = 40) age and sex matched controls classified into either low BMI (<= 25 kg/m^2) n=23 or higher BMI (>28 kg/m^2) n=23, 23–35 years old Irish (N= 85) and Indian (N=1)	Cross-sectional	16S rRNA gene amplification of the V4 region	PA assessed by validated EPIC-Norfolk questionnaire	187-food item FFQ	• Athletes α-diversity was higher compared to the high BMI (Shannon index, Simpson), or both control groups (Phylogenic diversity, Chao1, Observed species $p<0.05$. • Protein intake positively correlates with α-diversity (Shannon index, Simpson, Phylogenic diversity, Chao1, Observed species)(correlation coefficients 0.24-0.43, $p<0.05$) • Protein accounted for more of total energy intake among athletes (22%) compared to low BMI (16%) and high BMI (15%) controls • Athletes consumed more protein as a percentage of total energy compared to controls
(Estaki et al. 2016). **Country unknown**	N=39 Healthy males (n=22) and females, 18–35 years old Ethnicity not stated	Cross-sectional	16S rRNA gene amplification of the V3 and V4 region	Cardiorespiratory fitness - Peak oxygen uptake (VO_2peak)	Single 24hr dietary recall	• Determined that diet was not a confounding factor. • Increasing VO_2 peak significantly correlated with increased α-diversity (species richness $p=0.011$, $R_{adj}^2 = 2.04$, coefficient estimate = 5.36, $t = 2.17$) • *Colstridiales, Roseburia, Lachnospiraceae* and *Erysipelotrichaceae* genera significantly, positively associated with VO_2 peak ($p<0.05$) (butyrate-producing members)

Study	Participants	Study design	Microbiota analysis	PA and cardiorespiratory fitness assessment	Co-variates	Results
(Bressa et al. 2017) **Spain**	N=40, Healthy premenopausal women; active (n = 19) and sedentary (defined by meeting or not meeting WHO PA guidelines respectively) 18–40 years old BMI 20-25 kg/m^2 Caucasian	Cross-sectional	16S rRNA gene amplification of the V3 and V4 region	PA measured by Acti -Sleep V.3.4.2 accelerometer	97 food item FFQ	• No significant difference in α-diversity between active and sedentary groups (Observed, Chao1, Shannon index) • F/B ratio did not significantly differ between groups • Significantly higher presence of families *Barnesiellaceae* (p=0.001) and *Odoribacteraceae* (p=0.009) in sedentary compared to active women • Active group consumed significantly higher fibre compared to sedentary group 30.9 vs 21.5 g/day respectively (p=0.005)
(Mörkl et al. 2017) **Austria**	N=106, Females; Anorexia nervosa patients (n = 18), athletes (n = 20) had taken part in a regular training schedule for at least seven hours per week and who had participated regularly in competition. Normal weight (n = 26), overweight	Cross-sectional	16S rRNA gene amplification of the V1 and V2 regions	IPAQ & MET/min	2x 24-h dietary recall	• Athletes had significantly higher α-diversity compared to anorexia nervosa participants (observed species p=0.038, Chao1 p=0.019), normal weight participants (Chao1 p=0.037) and obese participants (observed species p=0.012, Chao1 p=0.011)

Study	Participants	Study design	Microbiota analysis	PA and cardiorespiratory fitness assessment	Co-variates	Results
	(n = 22), and obese (n = 20) 18–40 years old Ethnicity not stated					
(Petersen et al. 2017) **USA**	N=33 Males (n=22) and females (n=11) Professional (n = 22) and amateur (n = 11) level competitive cyclists 19–49 years old Ethnicity not stated	Cross-sectional	Metagenomic whole genome shotgun sequencing and RNA sequencing	Self-reported exercise load (hours/week): 6–10; 11–15; 16–20; and 20+	Non-validated questionnaire on diet and alcohol intake	• No significant correlations between taxonomic cluster and being professional or amateur level cyclist • High relative abundance of *Prevotella* in cyclists training > 11 h/week • Equal protein, fat, carbohydrate; vegetarian; high complex carbohydrate; paleo; gluten-free in each group
(Barton et al. 2018) **Ireland**	N=86 Professional male rugby athletes, and healthy size, age and sex matched controls (n = 46), either low BMI (≥ 25 kg/m^2) n=23 or higher BMI (>28 kg/m^2) n=23 23–35 years old Irish (N= 85) and Indian (N=1)	Cross-sectional	Metagenomic whole genome shotgun sequencing	PA assessed by validated EPIC-Norfolk questionnaire. Serum creatine kinase levels were used as a proxy for level of PA	187-food item FFQ	• The gut microbiota of athletes was more diverse at the functional level, than both the low and high BMI control groups • Athletes had an enriched profile of SCFA's

Study	Participants	Study design	Microbiota analysis	PA and cardiorespiratory fitness assessment	Co-variates	Results
(Keohane et al. 2019) **Ireland**	N=4 Well-trained male athletes 26.5 +-1.3 years old Ethnicity not stated	Prospective, repeated measures, within subject report	Metagenomic whole-genome shotgun sequencing	Before, during and after a continuous, unsupported 33-day, 5000 km transoceanic rowing race	Baseline dietary habits measured with adapted version of Willet FFQ Daily record of food consumed during the pre-race period on "My Fitness Pal" mobile application. Food consumed during the race was recorded by a food consumption record. Subjects provided with rehydrated, freeze-dried rations each athlete consumed a similar ration.	• Microbial α-diversity (Shannon index) increased throughout the ultra-endurance event (in 3 or the 4 subjects. Note the 4th subject took antibiotics before the mid-race sample). In two of these athletes a partial reversion was observed and in the other a complete reversion to baseline α-diversity levels was observed at three months follow-up • Throughout the race an increase in *Dorea longicatena, Roseburia hominis* (butyrate producer) and unclassified members of the genus *Subdoligranulum* (butyrate producers) was observed in all four athletes • Throughout the race an decrease in *Bacteroides finegoldii* was observed in all four athletes
(Durk et al. 2019) **USA**	N=37, Healthy participants males (N=20) and females (N=17)	Cross-sectional	16S rRNA	VO_2max measured with symptom-limited maximal graded treadmill exercise test	7 day diet record with MyFitnessPal.com body composition measured with BodPod®	• VO_2max was associated with increased in F/B (r = .48, $p<0.003$) and accounted for ~22% of the variance. No other variables (i.e., diet, body composition, or other fitness measures) were significantly

Study	Participants	Study design	Microbiota analysis	PA and cardiorespiratory fitness assessment	Co-variates	Results
	25.7 ± 2.2 years old White (37.8%), Hispanic/Latino (21.6%), Asian/Pacific (21.6%), Two or more ethnicities (16.2%), Other (2.7%)					correlated to relative gut microbiota composition.
(Langsetmo et al. 2019) **USA**	N=373, Community-dwelling older males ≥ 65 years old Non-Hispanic white (87.9%), "other" (12.1%)	Cross-sectional	16S rRNA gene amplification of the V4 region	SenseWear Pro3 Armband (step count) PA scale for the elderly (Washburn et al. 1999)	Age, race, BMI, clinical center, number of chronic medical conditions, and library size (OTU data) The Block 98.2 MrOS FFQ (Boucher et al. 2006)	• No consistent pattern by phylum • PA not associated with α-diversity (Shannon index, inverse Simpson) • Participants with higher self-reported PA were associated with higher β-diversity (unweighted Unifrac) after adjustment for confounders (principal co-ordinate analysis = 0.007, *p*=0.003). • Compared to those who were less active, those who had higher step counts had higher relative abundance of *Cetobacterium* (β =0.45, FDR *p*=0.00) and lower relative abundance of taxa from the genera *Coprobacillus (*β =-0.29, FDR *p=*0.04*), Adlercreutzia (*β =-0.25, FDR *p*=0.05)*, Erysipelotrichaceae* CC-115 (β =-0.37, FDR *p*=0.04).

Study	Participants	Study design	Microbiota analysis	PA and cardiorespiratory fitness assessment	Co-variates	Results
(Grosicki et al. 2019) **USA**	N=1 Male, world class ultra-marathon runner 32 years old Ethnicity not stated	Case study	16S rRNA gene amplification of the V4 region. Stool samples were obtained at 4 time points; 21 weeks and two weeks before and 2 hours and 10 days post the ultra-marathon)	Daily training log using Suunto Ambit3 Peak GPS watch VO_2 max	Weight, Body composition (DXA)	Two hours post-race • α-diversity (Shannon Diversity Index) increased from 2.73 to 2.80 and F/B ratio increased from 4.4 to 14.2 • Increases in relative abundance of bacterial genera *Veillonella* (+14,229%) and *Streptococcus* (+438%) • Decreases in relative abundance of *Alloprevotella* (−79%) and *Subdolingranulum* (−50%)
(Hampton-Marcell et al. 2020) **USA**	N=13 Male (n=5) and female division I NCAA collegiate swimmers 18-24 years old Ethnicity not stated	Longitudinal	16S rRNA V4 amplicon sequencing	Self-reported swimming distance and duration (hours) of daily practice during peak training through their in-season taper	BodPod®, skinfold thicknesses, weight	• α-diversity positively correlated with swimming yardage per week, decreasing with decreased training volume; Shannon index (R=0.43, $p<0.01$), inverse Simpson index (R=0.46, $p<0.01$). • Spearman rank correlations showed a significant reduction ($p<0.05$) with decreased training volume in *Coprococcus* (R=−0.25) and *Faecalibacterium* (R=−0.33).

[a]FFQ; Food frequency questionnaire, [b]EPIC; European Prospective Investigation of Cancer, [c]BMI; Body mass index, [d]WHO; World Health Organization, [e]IPAQ; International PA Questionnaire, [f]F/B; *Firmicutes to Bacteroidetes* ratio, [g]MET; Metabolic equivalent, [h]SCFA; Short-chain fatty acid, [i]DXA; Dual-energy x-ray absorptiometry, [j]BodPod®; an Air Displacement Plethysmography system, [k]FDR = false discovery rate adjusted. PA; physical activity.

2.5 Overall summary, conclusions and future research

PA and diet are widely acknowledged in the literature for their relationship with metabolic health. However, our way of thinking about PA, diet and health should not be stagnant. The growing appreciation for the reciprocal exchange between PA, diet and the gut microbiota may help improve our understanding of their combined association with metabolic health.

Dietary patterns are continually changing and we know diet is directly associated with metabolic health in complex ways that vary among individuals, cultures and geographical location. Therefore there is a need for further cross-sectional research to gather valid information about dietary intake and the relationship with PA and metabolic health (Imamura et al. 2015). This will assist in allowing clear direction when carrying out more expensive , timely trials investigating diet, PA and metabolic health to determine the most predictable outcomes for individuals' metabolic phenotypes.

Lastly, if disruption to the gut microbiota milieu does contribute meaningfully to the rapid rise in NCD's, specifically the global epidemic of obesity, this area warrants further investigation where both diet and PA are carefully considered. Specifically, research on diet and the gut microbiota should continue to be the backbone of this research, however, it is important that the independent and combined effect of PA behaviour supplement this work.

Although research on the gut microbiota and PA has gained traction over the past few years, human research in this field is in its infancy, and the application to human biology calls for urgent attention. Population research needs to continue to assist in identifying the specific PA patterns, including the duration of PA that play a role in shaping the microbiota. It is evident that research needs to continue in a wide range of humans including varying cultures, geographical locations, ethnic groups, ages and gender to identify the features of the gut microbiota that are influenced by PA and the carry-on effect to metabolic health. It is well recognised that all ethnicities can have obesity. It is important that minority ethnicities, especially those with high prevalence of obesity are included in future research. This will allow clear direction when carrying out more expensive, timely trials investigating diet, PA and the gut microbiota, to quantify the anti-inflammatory and metabolic effects to determine the most predictable outcomes to the host metabolic phenotype. Ultimately, this research will allow the limited public health purse to be better directed to allow policy makers to implement effective obesity and NCD prevention programmes, to help narrow the gap in health disparity. Given NZ has one of the highest rates of metabolic disease per capita, with two populations residing

in the country with markedly different metabolic risk (New Zealand Ministry of Health 2016); namely NZE and Pacific, it offers an ideal setting for this research.

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Chapter 3: Methodology

3.1 Study design

This PhD research is part of the HRC funded PRedictors linking Obesity and gut MIcrobiomE (PROMISE) study, conducted in Auckland New Zealand (NZ) between July 2016 and September 2017 (Table 3.1). The PROMISE study is a cross-sectional study which aimed to characterise the gut microbiota and metabolic profile in two populations with different metabolic disease risk, Pacific women (who have a high risk of obesity) and European women (who have a moderate risk of obesity) (Ministry of Health New Zealand, 2021), and different body fat profiles (normal and obese). Comparisons between Pacific and NZ European (NZE) women, who are known to vary the most in terms of physical, ethnic-cultural, and socioeconomic characteristics in NZ, were carried out to assess whether findings are different between groups. The PROMISE study involved the assessment of diet, taste perception, sleep, PA and SB and investigated the interactions between the gut microbiota and its impact on obesity, metabolic markers, and endocrine regulators. The **aim** of this thesis research was therefore to describe the PA and SB, and to explore the associations of PA and SB with body composition, dietary intake, biomarkers of metabolic health, and gut microbiota composition in these women. Therefore the current study only focused on the assessment of diet, PA and SB and their association with obesity, metabolic markers, and endocrine regulators. And lastly investigated the interactions between the gut microbiota and PA to determine if PA is a confounder in the relationship between the gut microbiota, obesity, metabolic markers, and endocrine regulators.

Detailed standard operating procedures and protocols were developed for all methodologies used in the study. All data were original to the PROMISE study, i.e. no existing or third party source was used. The full methodology of the PROMISE study is described elsewhere (Kindleysides et al., 2019) (see appendix 1).

Table 3.1 PROMISE Study funding details

PROMISE Study	
Funder	Human Research Council, New Zealand
Grant reference number	HRC Ref ID#: 15/273
Project name	PRedictors linking Obesity and gut MIcrobiomE (PROMISE) Study
Project description	Cross sectional
Primary Investigator	Professor Bernhard Breier
Related policies	PROMISE Study, School of Sport, Exercise and Nutrition, College of Health, Massey University

3.2 Participant recruitment and screening

Healthy NZ women who were either Pacific (people who identify with a Pacific ethnicity (eg Samoan, Tongan, Fijian) with or without other ethnicities, residing in NZ) (Stats New Zealand, 2015) or European (affiliated from different European nations) aged between 18 and 45 years, who reside in NZ, were invited to take part in the PROMISE study. Recruitment utilised a multi-media approach including print, web-based advertisements such as Facebook community pages or public figure pages (well-known NZ nutritionists, etc), work or university email lists, or other social media sources as well as person-to-person.

. Participants were screened for eligibility by completing a screening questionnaire, to screen for age, ethnicity, menstrual status, general health, dietary restrictions and body mass index (BMI), based on self-reported weight and height. This was conducted in person, via the phone or as an online, self-administered questionnaire (hosted by SurveyMonkey Inc, 2017©). Completed questionnaires were then reviewed by researchers. Women who had a BMI within the predefined normal or obese BMI ranges (BMI ≥ 18.5 to < 25.0 kg/m^2 and ≥ 30.0 kg/m^2, respectively) and met the below inclusion/exclusion criteria were invited to participate, with the aim of recruiting approximately equal numbers in each BMI and ethnic group.

Inclusion criteria were; aged 18 to 45 years, being post-menarche and pre-menopausal (as defined by regular menstrual cycles over the last year), ethnicity (self-identified Pacific ethnicity and having at least one parent of Pacific ethnicity (no minimum time for living in NZ was required), or self-identified as European ethnicity, with both parent of European ethnicity, and having themselves lived in NZ for a minimum of 5 years), willingness to comply with study requirements, and self-reported being generally healthy. Exclusion criteria were, self-reported BMI outside of the predefined normal or obese BMI ranges, pregnant or lactating, presence of any diagnosed chronic illness (e.g. type 2 diabetes and cardiovascular disease),

previous bariatric surgery, severe food allergies, medication that could interfere with appetite or the immune system (e.g. appetite suppressants and corticosteroids), current smoker, severe dietary restrictions or avoidances (e.g. vegan), and antibiotic use during the last month.

It is important to note that although our main target was to recruit participants with a normal BMI (18.5-24.9 kg/m^2) and an obese BMI (≥30.0 kg/m^2), the PROMISE study also recruited an additional 54 participants in the overweight BMI (25.0-29.9 kg/m^2) range and one participant with a BMI <18.5 kg/m^2. The over- and under-weight BMI groups were included in the PROMISE study (and this study) because, 1) some participants had incorrectly assessed their own height and weight before they arrived at the human nutrition research unit, and 2) to offset the enormous difficulties of recruiting normal BMI Pacific women.

In recognition that people with the same BMI can have substantial heterogeneity of body fat profiles and metabolic disease risk (Dickey et al., 1998; Kramer et al., 2013; Oliveros et al., 2014), for data analysis, participants were subsequently classified into two groups based on BF%: low-BF% (<35%) and high- BF% (≥35%) (Fitch and Bays, 2022). BF% cut-offs were derived from the American Association of Clinical Endocrinologists and American College of Endocrinology guidelines (obesity in women > 35%) (Dickey et al., 1998; Oliveros et al., 2014; Jo and Mainous, 2018). In 2022 cut-points were formalised according to the Obesity Medicine Association (obesity in women >35%) (Fitch and Bays, 2022).

The chosen cut-point for categorising low-BF% (<35%) vs high-BF% (≥35%) was confirmed after carrying out analysis within our population comparing BF% with BMI. In particular, scatter plots, graphing BMI vs BF% with a line of best fit suggested using 35% BF as our cut-point. Using this cut point only five participants with a BMI <25 kg/m^2 were in the >35% BF group, suggesting potential misclassification in terms of BMI was minor.

3.3 Ethical approval

The study was approved by Health and Disability Ethics Committees Ethics committee (HDEC, reference: 16/STH/32) and conducted in accordance with the Declaration of Helsinki.

3.4 Ethical considerations

3.4.1 Informed consent

All eligible participants were sent (via email, or given in person) an information sheet, about the study before they agreed to take part (appendix 3). The information letter contained the study co-ordinator’s phone number to contact if they had additional questions. Participants were given as much time as they needed to read the information sheet and discuss with whānau

before deciding if they would like to participate in the study. All participants, provided written informed consent prior to participation (appendix 3).

3.4.2 Right to withdraw

Participants were informed that they did not have to answer a question or carry out any procedure if they did not wish and that they had the right to withdraw from the study at any point.

3.4.3 Anonymity

Access to participant personal details was restricted; only the primary investigator (Professor Bernhard Breier), the study co-ordinator and the three PhD students on the PROMISE study had access to participants details. In order to maintain anonymity all participants were assigned a unique identity code which was used for all questionnaires and data forms.

3.4.4 Confidentiality

All participant documents were kept in a secure lockable filing cabinet to protect confidentiality. Participants details were held in a password protected computerised database for record keeping and to enable participants to be contacted. All other participant documents were stored in a separate password protected database that contained no personal details about the respondents.

3.4.5 Risk analysis

The risks involved in taking part in the study were low and the risk to research personnel was minimal.

3.4.6 Risks to participants

Prior to eligible participants consenting to the study, a further verbal explanation was given by researchers. No medical treatment was involved in the study and none of the study tests were considered dangerous to health, therefore no adverse effects were expected.

Participants underwent a dual-energy x-ray absorptiometry (DXA) whole body scan and blood sampling as part of this study. The exposure to x-rays has been calculated to be no more than the exposure experienced during an overseas flight. Blood sampling was conducted by trained phlebotomists in order to minimise risk of bruising, bleeding or discomfort.

The research team was very experienced working with women of various body fat profiles and the Pacific research team members (specifically, the Pacific liaison nurse) ensured all processes were culturally appropriate.

3.4.7 Risks to researchers

All DXA operators were fully trained and certified. All blood samples were taken by trained phlebotomists. All researchers followed standard infection control guidelines (Ministry of Health New Zealand, 2022), wore protective disposable gloves and lab coats when handling blood and faecal samples. Researchers processing and handling blood and faecal samples were previously immunised for hepatitis B.

3.4.8 Koha

Participants received a $100 Pak n Save or petrol voucher as well as their basic anthropometric and blood measurements, to thank them for their time and commitment to the PROMISE study.

3.5 Study Procedures

Eligible participants attended the Massey University Human Nutrition Research Unit in Albany, Auckland on two occasions, 11-14 days apart. Participants travelling from South Auckland were offered free transportation with the study Pacific liaison nurse. Study centre visits were performed on weekends and weekdays.

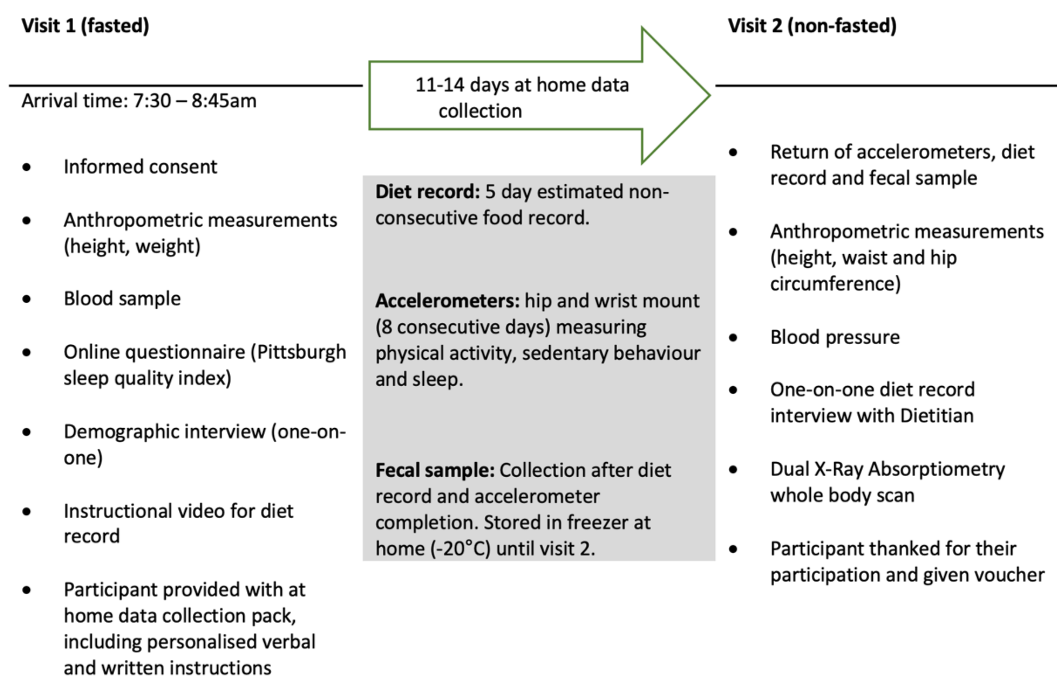

Figure 3.1 Overview of the study visits and at home data collection.

3.5.1 Anthropometry

All anthropometric measurements were conducted using the International Society for the Advancement of Kinanthropometry (ISAK) protocol (Stewart A, 2011). All research staff conducting these measurements were level 1 ISAK trained.

Body weight was measured in a fasted state on calibrated electronic scales (Sauter platform scale E1200, GmbH, Germany) that measured to the nearest 0.01 kg. Researchers ensured participants were wearing only one layer of light clothing, removed footwear, socks, hats and any jewellery they were wearing. Participant's height was measured to the nearest millimetre with a calibrated Harpenden stadiometer. Researchers aligned participants head in the Frankfort plane and positioned their heels and back against the vertical backboard of the stadiometer. Participants were asked to maintain this body position and deeply inhale. Researchers then lowered the stadiometer headboard until it rested firmly on the head of the participant. Height measurement was then read at eye-level from the vertical scale of the stadiometer. With the participant in a relaxed standing position with their arms folded across their chest and feet comfortably apart, waist and hip circumferences were measured to the nearest 1 mm. Measurements were taken at the end of normal expiration, against participants skin, using a narrow, flexible, stretch resistant, anthropometric tape measure (Lufkin W600PM). Waist measurements were taken at the narrowest part of the waist and hip measurements at the widest part of the buttocks.

For height, body weight, waist and hip circumference, two measurements were taken at each site and recorded. A third measurement was taken only if the second measurement was not within 1% of the first. The mean value was recorded if two measurements were taken, and the median if three measurements were taken. Actual BMI (kg/m^2), used for data analysis, was calculated using measured weight and height.

3.5.2 Body composition

Body composition measurements, including total and regional (android, gynoid, trunk and visceral) fat mass, and total lean mass, were assessed using DXA (Hologic QDR Discovery A, Hologic Inc, Bedford MA) (APEX V. 3.2 software), by accredited researchers (Australian and NZ Bone Mineral Society clinical densitometry accreditation) (Kindleysides et al., 2019). Before scanning, participants emptied their bladder and were asked to wear tight fitting clothing, remove footwear, glasses and jewellery. They were also asked if they were pregnant or had a pacemaker, if the participant responded "yes" they were not scanned. (Kindleysides et

al., 2019). If a participants body did not fit completely within the narrow screening parameters of the DXA, two scans were taken. One scan was including the right arm and the other was of the participant's whole body except the complete right arm. The complete right arm data were then used to replace the incomplete right arm on the whole body scan. For the first scan the participant was positioned on the scan table so that the entire left side of their body fitted on the table. Any overflow of the right arm was directed off the right side of the scan capture, and care was be taken that it was only the right arm and no other body parts that were incomplete from the first scan. The second scan was performed immediately after the first scan ensuring the whole right arm was included in the second scan. DXA-measured total BF% was used to categorise participants as low-BF% (<35%) or high- BF% (≥35%).

3.5.3 Blood pressure and heart rate

At clinic visit two, following a 10-minute seated, resting period, systolic and diastolic blood pressure and heart rate were measured in the supine position, with a digital blood pressure monitor (Omron HEM-907, Omron Healthcare Inc). Researchers used the most appropriate arm cuff size (22-32 cm or 32-48 cm). Three consecutive measurements were taken at one minute intervals. As the first measurement is considered the most unreliable, the mean of the second and third measurements were used for analysis (Egan et al., 2010).

3.5.4 Demographic information

Standardised face-to-face interviews with a researcher captured a comprehensive range of demographic, work and health information, including any medications participants were taking. NZ Deprivation Index 2013 (NZDep2013) (Atkinson J, 2014) was used in this study as a proxy for socioeconomic status. NZDep2013 is derived from participants geographical area of residence and it combines census data relating to home ownership, housing, qualifications, income, employment, access to transport, communications, and family structure. A NZDep2013 score of one represents the areas "least deprived" and 10 the areas "most deprived".

3.5.5 Dietary assessment

Dietary intake was assessed for energy, macro- and micronutrient intakes using five-day estimated food records (5DFR) (Gibson, 2005). Researchers pre-allocated non-consecutive days for participants to complete food records, in order to obtain an even spread of days of the week throughout the study population, this included at least one weekend day. Estimated, rather than weighed food records were used to improve adherence and to reduce participant burden (Gibson, 2005, Livingstone and Black, 2003). Participants received verbal training and also listened to a 15-minute food record demonstration video (developed by nutritionists and NZ

Registered Dietitians at Massey University), providing detail on how to complete the food record with sufficient detail and precision. Participants were provided with a standarised food portion booklet (developed by NZ registered Dietitians from Massey University), accompanied by images of standard household food measures (e.g. metric cups and spoons), to help estimate portion sizes.

At clinic visit two, when food records were returned, NZ Registered Dietitians meticulously reviewed participant's food records. Dietitians then carried out a comprehensive, in-person, one-on-one interview with participants, to clarify portions of foods and beverage consumed, cooking methods, brands of food and beverage products reported and any other ambiguities. The food portion booklet, standard household food measures and web-based tools were used to confirm specific portion sizes and brands consumed, respectively. This interview was critical in ensuring accurate dietary data, with the appropriate level of detail were captured (Kruger et al., 2012).

Nutrient analysis of the food record data was performed using the Foodworks 9 (Xyris Software Pty Ltd) dietary analysis software, for chapter 1 and 2 and Foodworks 10 (Xyris Software Pty Ltd) for chapters 3 and 4, which uses FOODfiles 2016 (developed by the NZ Institute for Plant & Food Research and the NZ Ministry of Health) as a reference food composition table for analysis. If the food or beverage was not in FOODfiles 2016, the Xyris database AusFoods 2017 and AusBrands 2017, which are based on the Australian food composition databases AUSNUT 2011-13 (developed by Food Standards Australia New Zealand) were used.

Independently two researchers entered the 5DFR into Foodworks (Xyris Software Pty Ltd). To confirm reliability, a third researcher checked data entry. A dietary data dictionary and standard operating procedure were both developed to ensure consistent data entry.

All exported data were extensively reviewed for plausibility of intake, by NZ registered dietitians. Researchers considered energy intakes between 2100 kJ/day and 14600 kJ/day to reflect what the participants reported plausibly consuming (Willett, 2012). Additionally, data was checked for plausibility, where those who had an unrealistic high or low energy intake, were asked to clarify their intake (Ministry of Health New Zealand, 2011). After reviewing exported data, reported intakes below or above these cut-offs were classified as misreporting and excluded from data analysis.

Nutrient intake estimates were only derived from food and beverages, intake from dietary supplements were excluded (with the exception of supplements that provided energy e.g. sports

protein drink). The nutrient list included: Total energy (in kJ.day^{-1}), total carbohydrate, starch, sugar, total dietary fibre, protein, total fat, saturated fat, monounsaturated fat, polyunsaturated fat, and alcohol. The following micronutrients were also analysed due to their importance for women's health; iron, B12, iodine and calcium (Bartley et al., 2005). Total energy intake was calculated by summing energy contribution from all macronutrients including total dietary fibre, and alcohol (all in g/day). The percentage of energy intake from all macronutrients was calculated.

3.5.6 Physical activity and sleep

To objectively measure physical activity (PA) and sedentary behaviour (SB), participants wore a tri-axial w-GT3X accelerometer (Actigraph, Pensacola, FL) (Actigraph LLC, 2016) on their nondominant hip. To objectively measure sleep onset and wake times and an Acti-Watch (Micro Motionlogger®) on their non-dominant wrist, which are the standard placements for measuring movement (Ward et al., 2005) and sleep (Ancoli-Israel et al., 2003), respectively. Both devices were initialised on the same computer, on the day of the participants first clinic visit and were initialised to start recording at the same time (12 noon on the day of clinic visit one).

In general, when measuring PA the monitor should be programmed to begin recording at midnight on the first day it is to be worn by the participant, however for recording sleep the monitor should be programmed to begin recording at mid-day, and should be set to record for the maximum amount of time in case the monitoring period needs to be extended to make up for days of non-wear (Matthews et al., 2012b). Because the wider PROMISE study was measuring PA as well as sleep, and participants were coming into the clinic in the morning a mid-day to mid-day recording format was used, to maximise the amount of data gathered. Thus only capturing half a day's PA behaviour on day one and eight.

Both devices were set to record epoch length of one minute. Participants were instructed to wear both devices continuously (24-hour protocol) for the following eight days, which included at least one weekend day, except while bathing or participating in water activities such as swimming (Jaeschke et al., 2018). During the eight day collection period, participants completed a daily PA and sleep diary. Each day participants recorded 1) sleep onset and 2) sleep end times were recorded for any sleep, including naps $\geq$ 10 minutes duration, 3) any time the participant removed and put back on accelerometers and what they were doing in the time they were not wearing the accelerometers (e.g swimming, bathing).

Participants were asked to press the ActiWatch "event" button when they went to bed and turned off their light to sleep, and when they woke up. This was to mark sleep onset and wake time in the ActiWatch (WatchWare Software (Version 1.94.0.0 and higher).

Participants also completed the previously validated; Pittsburgh Sleep Quality Index questionnaire (PSQI) (Buysse et al., 1989), hosted by Survey Monkey© (SurveyMonkey Inc, 2017). The questionnaire was online, self-administered, forced-choice, multi-choice, and designed to take 10 minutes to complete. The PSQI covers seven areas in which sleep problems occur; 1) subjective sleep quality, 2) sleep latency, 3) sleep duration, 4) sleep efficiency, 5) sleep disturbance, 6) use of sleep medication and 7) daytime dysfunction. The sum of scores for these seven sleep areas equals one global score, ranging from 0 to 21, a lower score indicates a healthier sleep quality.

3.5.7 Actigraph data processing

The hip mounted accelerometer data was imported into and processed in Acti-Life® software (version 6.13.3). All accelerometer epochs that occurred during sleep periods were identified using the sleep diaries and confirmed using ActiWatch accelerometer. Sleep times were subsequently removed, leaving only epochs that occurred during waking hours for analysis. To confirm the accuracy of sleep diaries a trained researcher visually inspected participants' individual ActiWatch download graphs (using WatchWare software (version 1.94.0.0) concurrently with their sleep diaries. If the sleep diary stated the participant was sleeping and either; 1) the participant had pressed the sleep onset/wake button OR 2) the ActiWatch graph visually indicated the participant was sleeping, this was confirmed as a correct sleep period. If no time was written in the sleep diary the ActiWatch time was used.

Non-wear time was defined as ≥ 60 consecutive minutes of zero epoch counts, with allowance of two minutes of counts between zero and 100. Participant's data were considered valid if they wore the accelerometer for ≥ 12 hours per day (Matthews et al., 2012a), on ≥ four days, including one weekend day (Tudor-Locke et al., 2012). For participants that Actilife marked as having insufficient valid accelerometer data, a researcher looked at their individual w-GT3X Actigraph outputs concurrently with their accelerometer wear diaries, to determine if they had been correctly categorised as not meeting the valid wear time requirements. It was determined that all these participants were not wearing the accelerometer for either the appropriate length of time and/or days. These participants were excluded from analysis involving PA. The remaining epochs were categorised into time spent in the following different daily levels of PA (counts/min), using widely used and validated cut-points (Troiano et al., 2008); moderate PA

(2020-5998 counts/min) and vigorous PA (≥ 5999 counts/min), moderate to vigorous PA; a composite measure of moderate and vigorous PA (≥2020 counts/min), light intensity PA (100 - 2019 counts/min), or SB (zero – 99 counts/min). Vector magnitude mean activity count (counts/min) was used as an indicator of total daily PA.

Once data was “scored” in Actilife (marked as the aforementioned movement behaviours) the “calculate” tab was selected and data was exported into Excel. Data processing differed slightly between research chapter 1 and the other three research chapters (2-4). After completing research chapter 1, it was clear the population was very sedentary, therefore in research chapter 2 it was decided to look at SB’s (e.g. weighted median sedentary bout length, maximum sedentary bout length) as well as total time spent in SB. Actilife does not automatically calculate weighted sedentary bout length. Therefore it was required to calculate manually. Actilife data processing method used for chapter 1 does not provide the daily breakdown of specific SB’s, it only provides the mean of all valid days in its data output. Using the “subject log diary” function in Actilife, allowed the daily breakdown of specific SB’s, so weighted median bout length could subsequently be calculated.

For analysis of all PA and SB data, it was specified within Actilife, to analyse data from mid-night to mid-night. Data from the first and last days (days of partial wear) of monitoring were excluded from analysis due to incomplete data. Further, this allowed provision for subject reactivity (participants increasing PA as they know they are being monitored) on the first day of wearing (Baumann et al., 2018). For research chapter 1, data from the first and last days of recording were manually removed after data had been exported into Excel. For research chapters 2-4, accelerometer start and end dates (to be analysed) were entered into “subject log diaries” which were then uploaded into Actilife, this allowed data from the first and last days of recording to be removed before data was exported into Excel (software used for the processing). The two data processing methods resulted in minimal difference in data sets (e.g. ≤ 3% in SB, ≤ 2% in light PA and ≤ 8% in MVPA) (Table 3.2).

Table 3.2 Difference in accelerometery data sets between research chapter 1 and 2-4, by BF% group and ethnicity

Accelerometery	**Research chapters 2-4 data set**				**Research chapter 1 data set**			
	Pacific		*NZE*		*Pacific*		*NZE*	
	<35% body fat	≥35% body fat	<35% body fat	≥35% body fat	<35% body fat	≥35% body fat	<35% body fat	≥35% body fat
Light physical activity (min/day)	310	344	328	320	314	342	328	321
Sedentary behaviour (min/day)	615	591	563*	579*	596	575	546	560
MVPA (min/day)	24	19	39	30	26	19	39	31

*significant difference between research chapters 2-4 data set and research chapter 1 data set (two tailed paired T Test)p=<0.001

SB variables includes (technical descriptions in Table 3.3): Daily average of sedentary breaks (minutes); average length of sedentary breaks (minutes); maximum length of sedentary breaks (minutes); fragmentation Index; average length of sedentary bouts (minutes); maximum length of sedentary bouts (minutes); daily average of sedentary bouts (minutes); weighted median sedentary bout length (minutes); total time spent in SB per day (standardised to a 16 hour day). Total SB was standardised to a 16 hour day to equalise the different intervals participants actually wore the accelerometers; for example, if a participant wore the accelerometer for 10 hours and recorded 300 minutes of sedentary time, this would be standardised to 480 minutes in a 16-hour day.

Table 3.3 Description of technical sedentary behaviour terminology

Terminology	Description
Sedentary bout	≥10 minutes continuous sedentary time, with no interruption allowed.
Break in sedentary bout	Transition (lasting at least 1-minute) in accelerometer count from <100 cpm to >100 cpm in between two sedentary bouts.
Weighted median sedentary bout duration	The length of the sedentary bout corresponding to 50% of total, daily, accumulated sedentary time. For example, if a participant recorded 10 hours of sedentary time, after ordering bouts cumulatively from smallest to largest, the weighted median sedentary bout length represented the length of the bout that contained the five-hour time point. A higher weighted median bout length, indicates less interrupted SB (Chastin et al., 2015).
Fragmentation index	An index of breaks in sitting time; the ratio of the number of sedentary bouts (≥10 minutes) divided by total sedentary time. A higher fragmentation index indicates more interrupted SB.

3.5.8 Blood biomarkers

Fasted participants attended the clinic between 7:30am and 9:00am (overnight fasting time was recorded;10-15 hours). A tourniquet was applied to the arm before venepuncture and the time blood was drawn was recorded. Trained phlebotomists drew a maximum of 30 ml of blood into four vacutainers (Ethylenediaminetetraacetic acid (EDTA) 10 mL vacutainers (Becton Dickinson) and silicone coated (with clot activator) vacutainer tubes), to obtain serum and plasma for analysis of metabolic markers. An aliquot of EDTA whole blood was frozen at -80°C immediately following blood collection (for HbA1c analysis). The serum vacutainer was left to stand at room temperature (18°C) for between 30 and 60 minutes, for clot formation before centrifugation (Becton Dickinson). For endocrine regulators, an additional plasma sample (2 mL Becton Dickinson vacutainer P800 EDTA, aprotinin, and dipeptidyl peptidase IV) was collected. The latter and the remainder of the samples were placed on wet ice (kept at 4°C) until centrifugation. Within one hour of sample collection all EDTA vacutainer tubes (including the remainder of the whole blood) were centrifuged at 3500 rpm for 15 minutes at

4°C. Aliquots of plasma and serum were transferred into pre-labelled 1.5 mL microcentrifuge tubes (Eppendorf® safe-lock PCR clean tubes, Hamburg, Germany) and cryovials (Cryo.S Greiner Bio-One, GmbH) and stored immediately at −80° C (Kindleysides et al., 2019).

Blood samples were analysed for a range of biomarkers, inflammation markers and endocrine regulators. Plasma levels of Glucagon-like peptide-1(GLP-1), Ghrelin, Leptin, Interleukin 6 (IL-6) and Tumor Necrosis Factor Alpha (TNFα) were analysed by Plant & Food Research Mt Albert, Sandringham, NZ (fully-accredited laboratory with IANZ to the ISO 15189 standard), using the MILLIPLEX MAP Human Metabolic Hormone Magnetic Bead Panel 96-Well Metabolism Multiplex Assay (Millipore, USA, Cat # HMHEMAG-34K). The Plasma Peptide YY (PYY) (total) assay was conducted using a Human PYY (total) 96 well plate ELISA (Millipore, USA, Cat # EZHPYYT66K). Analytes were assayed in duplicate, and plates were read with the use of the Bio-Plex® 100 Analyzer System (Bio-Rad). The following biomarkers had reasonable or good percentage of values above the limit of detection (>LOD); ghrelin 85.2 % >LOD, leptin 98.4% >LOD, TNFα 98.4% >LOD. However, GLP-1 had marginal percentage of values (64.0%) >LOD and IL-6 had poor percentage of values (23.3%) >LOD. Consequently IL-6 was not used for further analysis.

The remainder of blood samples were analysed by the Liggins Institute, University of Auckland in NZ. Serum levels of glucose (enzymatic UV method), total cholesterol (chol), Non-esterfied fatty acids (NEFA), triglycerides (Trig), high density lipoprotein (HDL), low density lipoprotein (LDL) and C-Reactive Protein (CRP), were measured using a Hitachi e11 automatic electronic analyser (Hitachi High Technologies Corporation, Tokyo, Japan), and kits supplied by Roche Diagnostics (Mannheim, Germany). Chol (Kit lot # 00276302), NEFA (Kit lot # AN501, AN502, AP177, AP178) and Trig (Kit lot #00279798), were measured using enzymatic colorimetric, whilst homogenous enzymatic colorimetric was used to measure HDL (Kit lot #00206423) and LDL (Kit lot #00209188). CRP was measured by Particle-enhanced Immunoturbidimetric (kit lot # 00267926), glucose was measured using Enzymatic UV (Kit lot #00337762). Serum insulin was measured by Electrochemiluminescence immunoassay method (ECLIA) (Kit lot #00215871) (Roche Diagnostics, Mannheim, Germany) using the Cobas e411 analyser (Hitachi High Technologies Corporation, Tokyo, Japan). Inter assay coefficients of variation for Chol, NEFA, Trig, HDL, LDL, CRP, glucose and insulin were 2.0, 3.4, 0.8, 6.1, 0.9, 3.3, 0.7 and 0.5 %, respectively.

EDTA whole-blood HbA1c levels were measured on a Hitachi c311 autoanalyser (Hitachi High Technologies Corporation, Tokyo, Japan), by turbidimetric inhibition immunoassay

(Roche Diagnostic, Mannheim, Germany). The inter-assay coefficient of variation for HbA1c analyses was 1.0%.

Insulin resistance was calculated using HOMA of insulin resistance (HOMA-IR) (HOMA-IR = (fasting plasma insulin (pmol/ml) x fasting blood glucose (mmol/L))/ 22.5) (Wallace et al., 2004).

3.5.9 Gut microbiota

Two separate faecal samples (from the same faeces) were self-collected, in the three days following removal of their accelerometer devices and completion of the 5DDR (9-14 days post visit one). Participants placed the provided kidney dish in the toilet to catch the faeces. They used the scoop that was inside the lid of two small screw top containers to collect the walnut sized faecal samples. Two larger containers (provided) were then filled with 2 cm of water and the two smaller containers were placed inside, so the small containers were surrounded by water. The date and time of faecal collection was written on both large containers. Participants then immediately placed the faecal sample into their household freezer (-20° C) until returned to the clinic. Participants were provided with an insulated chilly bag and an ice-pack, that they froze and placed in the chilly bag with their sample for maintenance of sample integrity during transport. Upon arrival to the research clinic a PROMISE researcher, immediately placed the faecal samples in the -80° C freezer for storage until analysis. Faecal samples were transported by World Courier© using dry ice to maintain temperature, to University of Otago, Microbiology department for microbiota analysis by Professor Gerald Tannock and Dr Blair Lawley.

3.5.10 Gut microbiota analysis

DNA extraction and sequencing: Following the kit protocol provided by the manufacturer (with the following modification: faecal samples were suspended in 1mL of TN150 buffer (containing 10 mM TRIS-CL pH 8.0, 150 mM NaCl)), DNA was extracted from 250 mg faecal sample (PowerSoil DNA isolation kit, Mo Bio, Carlsbad, CA, USA). The suspension was centrifuged at 14,600×g (3 min, 5°C) and then suspended in 700 µl solution from the PowerBead Tubes. The suspension was added back to the PowerBead Tubes and the standard protocol followed. DNA was eluted in 100 µl of elution buffer (warmed to 70°C) and stored at -80°C. A Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) were used to check quality and quantity of genomic DNA. The cleaned DNA was sent to New Zealand Genomics Ltd. (NZGL) to conduct shotgun metagenome sequencing.

Using an Illumina HiSeq 2500 instrument (Illumina, San Diego, CA, USA), NZGL prepared 384 Thruplex DNA libraries and carried out 2×125 bp paired-end sequencing across 24 lanes. To sequence libraries, a minimum of six HiSeq lanes were used. Multiple libraries were prepared for several samples, in order to test for library preparation and sequence run bias. An average of 13,150,561 (range 7,6940,894-17,081,755) reads were recovered for each sample. For quality control BBDuk (https://sourceforge.net/projects/bbmap/) was used to trim adapters, remove low quality reads and remove reads <100 bp after trimming. KneadData (http://huttenhower.sph.harvard.edu/kneaddata) was used as quality control to remove human genome reads (i.e., DNA) from bacterial reads, implementing the hg19 database. Sequence data used in this study will be deposited with the short-read archive (SRA).

3.6 Statistics and bioinformatic analysis

3.6.1 Bioinformatic analysis

Microbiota taxonomic profiles were created from DNA sequences using MetaPhlan 2.0 (version 2.6.0) according to default parameters (Segata et al., 2012). QIIME2 (version 2018.8, (Bolyen et al., 2018) (https://qiime2.org/) was used to analyse microbiota composition and α-diversity, using converted output tables from MetaPhlan 2.0. PERMDISP was used to measure group dispersion (Anderson, 2006). Previous work by the PROMISE study team characterised the composition of the participant's microbiota in terms of enterotypes (Renall, 2020). Three enterotypes were identified Table 3.4. Following the tutorial provided by EMBL (http://enterotyping.embl.de), enterotypes were predicted in R using the approach described in Arumugum et al. (Arumugam et al., 2011, Renall, 2020). To determine which species were driving enterotypes, differential abundance testing was carried out with Statistical Analysis of Taxonomic and Functional Profiles (STAMP) (Parks et al., 2014). Each enterotype was compared to all other samples using Welch's t-test using Benjamini-Hochberg for multiple testing correction.

Table 3.4 Description of Enterotypes identified by PROMISE study

Enterotype	**Predominately found in population**	**Characterised by abundance of microbiota bacteria**
Enterotype 1	Both Pacific and NZE women	Eubacterium rectale and Faecalibacterium prausnitzii (butyrate-producing bacterial species)
Enterotype 2	Pacific women	Bifidobacterium Bifidobacterium Lactobacillus ruminis (Lactic acid-producing bacterial species)
Enterotype 3	NZE women	Subdoligranulum species, Akkermansia muciniphila, Ruminococcus bromii, and Methanobrevibacter smithii

(Renall, 2020)

3.6.2 Statistical analysis

Statistical analyses were conducted using IBM SPSS software for Windows version 24.0 (SPSS Inc, Chicago, IL). For all analysis p values <0.05 were considered statistically significant. Normality of data was confirmed using histograms and Kolmogorov-Smirnov tests. Data that were not normally distributed were logarithmically transformed (*ln*) to ensure a normal distribution.

Descriptive statistical methods were used to summarise PA, SB, dietary intake, biomarkers and gut microbiota composition, α-diversity and enterotypes. Means and standard deviations (SD) were used to summarise all continuous data, except variables that were logarithmically transformed, which were presented as geometric means and geometric standard deviations (GSD). Frequencies (%) were reported for categorical variables. Comparisons of participant characteristics focused on the differences between BF% groups within an ethnic group (e.g., NZE low-BF% versus NZE high-BF%), and between ethnicities within a BF% group (e.g., NZE low-BF% versus Pacific low-BF%). Differences between ethnic and BF% groups were measured with one-way ANOVA, with Bonferroni post hoc correction.

Multivariate regression models were conducted to explore the association between PA, SB and diet, body composition and biomarkers of metabolic health, as well as the association between

PA, SB and gut microbiota composition. Stratified analyses was performed to assess effect modification. Due to NZE women being significantly older ($p<0.05$) and less deprived ($p<0.05$) than Pacific women and considering age and deprivation are negatively associated with metabolic health (Ministry of Health New Zealand, 2021), regression analysis was carried out controlling for age and deprivation index (NZDep2013) and when appropriate also adjusted for other potential confounders (e.g. BF% group, diet, sleep quality, PA). Independent variables were assessed for collinearity by assessing tolerance and the variance inflation factor.

Regression coefficients (β) obtained from log-transformed data (fasting insulin and CRP as well as gut microbiota variables *Erysipelotrichaceae, Rikenellaceae, Verrucomicrobiaceae, Veillonellaceae* and *Firmicutes:Bacteroidetes* ratio) represent relative differences and were expressed as a ratio (by using e^{β}). Analyses were conducted separately for NZE and Pacific participants.

Table 3.5 Summary of study measurements and methods

Measurement	Methods	Reference	Equipment	Outcomes
Body composition profile	Anthropometric measurements	(Ling et al., 2011)	Stadiometer, anthropometric tape measure (Lufkin W600PM), electronic scales (Sauter platform scale E1200, GmbH, Germany)	Linear body measurements – height, weight, waist and hip circumference, BMI (kg/m^2)
Body composition distribution	Dual XRay Absorptiometry (DXA)	(Boneva-Asiova and Boyanov, 2008)	Dual XRay Absorptiometry (DXA) (Hologic QDR Discovery A, Hologic Inc, Bedford, MA. with APEX V. 3.2 software	Body composition Total and regional fat mass
Metabolic heath biomarkers			Blood sampling to capture biomarkers (e.g. plasma glucose, insulin, HbA1c, lipids), inflammation markers (e.g. hs-CRP, IL-6, TNFα), endocrine regulators (e.g. GLP-1, ghrelin, leptin)	Biomarkers related to metabolic health (lipid profile, glucose control, inflammation, hormonal control)
Blood pressure and heart rate	Digital blood pressure monitor	(Ogedegbe and Pickering, 2010)	Omron HEM-907, Omron Healthcare Inc, using one of two arm cuff sizes (22-32cm or 32-48cm)	Blood pressure related to metabolic health
Dietary intake	5 day estimated food record	(Kruger et al., 2015)	Analysis using Foodworks9 2016 (Xyris Software, Australia) and Foodworks 10 2020 (Xyris Software, Australia)	Energy intake, macro- and micro-nutrient intake,
Physical Activity and sedentary behaviour	Accelerometer	(Pescatello)	WGT3X Actigraph	Objective movement behaviour - sedentary behaviours - levels of physical activity (light, MVPA) - Total physical activity (cpm)
Sleep behaviour	Accelerometer	(Micro Motionlogger®)	Acti-Watch	Objective sleep onset and wake times
	Pittsburgh sleep quality index	(Buysse et al., 1989, Roenneberg et al., 2007) (Sharma et al., 2006)	Hosted online via Survey Monkey	Global measure of sleep quality
	Sleep diary		Paper based, entered into Excel	Self-reported sleep onset and wake
Gut microbiota	DNA extraction & sequencing	(Leimena et al., 2013)	Illumina HiSeq 2500 instrument using Nextera using library preparation(NZ Genomics Ltd)	Relative abundance of bacterial species and α-diversity

3.7 References

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Chapter 4: Objectively measured physical activity is associated with body composition and metabolic profiles of Pacific and New Zealand European women with different metabolic disease risks

Abstract

Objective: To assess associations between physical activity (PA), body composition, and biomarkers of metabolic health in Pacific and New Zealand European (NZE) women who are known to have different metabolic disease risks.

Methods: Pacific (n=142) or NZE (n=162) women aged 18-45 years with a self-reported body mass index (BMI) of either 18.5-25.0 kg/m^2 or ≥30.0 kg/m^2 were recruited and subsequently stratified as either low (<35%) or high (≥35%) BF%, with approximately half of each group in either category. Seven-day accelerometery was used to assess PA levels. Fasting blood was analysed for biomarkers of metabolic health, and whole-body dual-energy x-ray absorptiometry was used to estimate body composition.

Results: Mean moderate-to-vigorous physical activity (MVPA; min/day) levels differed between BF% ($p<0.05$) and ethnic ($p<0.05$) groups: Pacific high- 19.1 (SD 15.2) and low-BF% 26.3 (SD 15.6) and NZE high- 30.5 (SD 19.1) and low-BF% 39.1 (SD 18.4). On average Pacific women in the low-BF% group engaged in significantly less total PA when compared to NZE women in the low-BF% group (133 cpm); no ethnic difference in mean total PA (cpm) between high-BF% groups were observed: Pacific high- 607 (SD 185) and low-BF% 598 (SD 168) and NZE high- 674 (SD 210) and low-BF% 731 (SD 179). Multiple linear regression analysis controlling for age and deprivation showed a significant inverse association between increasing total PA and fasting plasma insulin among Pacific women; every 100 cpm increase in total PA was associated with a 9% lower fasting plasma insulin; no significant association was observed in NZE women. For both Pacific and NZE women, there was an 8% reduction in fasting plasma insulin for every 10-minute increase in MVPA ($p\leq0.05$).

Conclusions: Increases in total PA and MVPA are associated with lower fasting plasma insulin, thus indicating a reduction in metabolic disease risk. Importantly, compared to NZE, the impact of increased total PA on fasting insulin may be greater in Pacific women. Considering Pacific women are a high metabolic disease risk population, these pre-clinical responses to PA may be important in this population; indicating promotion of PA in Pacific women should remain a priority.

4.1 Introduction

Obesity is a global public health problem. The prevalence in New Zealand (NZ) is among the highest in the world, with 31% of the population aged ≥15 years considered to be obese (body mass index (BMI) ≥30.0 kg/m^2, or International Obesity Task Force equivalent for 15-17 years) (Ministry of Health New Zealand, 2021). Obesity is described as an "excessive fat accumulation that may impair health" (World Health Organisation, 2016) and the World Health Organisation has defined standardised cut-points for overweight (BMI ≥25.0 and <30) and obesity (BMI ≥30.0). However for a given BMI, factors such as age, sex and ethnicity influence body fat percentage (BF%) and distribution, thus individuals with the same BMI may have different metabolic disease risks (Kramer et al., 2013, Oliveros et al., 2014). Global and NZ trends show a significantly greater rise in obesity prevalence among women, when compared to men, with major BMI gains between the ages of 20-40 years (The GBD 2015 Obesity Collaborators, 2017). Furthermore, the global obesity prevalence in women nearly tripled from 6% in 1975 to 15% in 2014 (NCD Risk Factor Collaboration, 2016); in 2008, this was projected to rise to 21% by 2025 (Kelly, 2008, NCD Risk Factor Collaboration, 2016). Alarmingly, current estimations of obesity among NZ women already surpass this figure, with 32% currently classed as obese (Ministry of Health New Zealand, 2021). Obesity disproportionally effects disadvantaged socioeconomic (Zhang and Wang, 2004) and ethnic groups (Hales CM, 2020). In NZ, adults living in the most deprived areas are 1.6 times more likely to have obesity compared to adults living in the least deprived areas. Obesity prevalence is highest among Māori (the indigenous population of NZ) (48%) and Pacific (70%) women (Ministry of Health New Zealand, 2021), which is of particular concern as obesity is associated with a substantial burden of disease (Wagner and Brath, 2012). The high prevalence of obesity among Māori may, among other reasons (e.g. deprivation), be due to the impact of colonisation, which has resulted in a loss of traditional food gathering practices and places, as well as the introduction of new foods (Gray, 2003, Ministry of Health, 2006). In addition, current individualistic approaches of western medicine to obesity management are not culturally aligned with Indigenous people's holistic health believes and cultural practices, hampering the effectiveness of public health interventions (Bell et al., 2017). Some of these factors may also play a role in the high prevalence of obesity among Pacific people in NZ.

Obesity reflects a state of positive energy balance and is associated with altered glucose and lipid metabolism that may lead to an increased risk of non-communicable diseases, such as type 2 diabetes (T2D), some cancers and cardiovascular disease (CVD) (Wagner and Brath,

2012). Hence, the long-term health impact of obesity is considerable, especially for women of childbearing age, as increased adiposity is also associated with increased obesity risk for the next generation (Eriksson et al., 2014).

Currently, physical activity (PA), together with diet, are the cornerstones of obesity intervention and prevention. Many of the health benefits associated with PA are mediated through its ability to improve glucose and lipid metabolism (Timmermans et al., 2006). Current PA guidelines include promoting ≥30 minutes of moderate (or greater) intensity activity on at least five days of the week (U.S. Department of Health and Human Services, 2018, Ministry of Health New Zealand, 2020). However, a large review study (1.9 million participant across 168 countries) found the global age standardised prevalence of insufficient PA to be as high as 27.5% (Guthold et al., 2018). Furthermore, a higher prevalence of women were not meeting the PA guidelines compared to men (31.7% and 23.4% respectively). In NZ, only 48% of women met PA recommendations in 2019/20 (Pacific and NZE women 39% and 51% respectively) (Ministry of Health New Zealand, 2021). In agreement with global trends, NZ women were less physically active when compared to NZ men (55% met PA recommendations) and NZ adults living in the most deprived areas are least likely to meet these PA guidelines (Ministry of Health New Zealand, 2021). Even more concerning, is that 25% of Pacific and 14% of NZE women fail to perform even 30 minutes of moderate PA per week, classifying them as inactive (Ministry of Health New Zealand, 2021). It is therefore not surprising that physical inactivity is currently classified as the fourth leading risk factor for global mortality (World Health Organisation, 2017). Recent research suggests that total PA volume accumulated throughout the day may be as important as time spent in moderate-to-vigorous PA (MVPA) for lowering markers of metabolic disease risk (e.g., fasting insulin, high density lipoprotein (HDL)) (Ekelund et al., 2007, Swindell et al., 2018). To date, research on PA and its association with metabolic health has focused on healthy subjects (Healy et al., 2008, Knaeps et al., 2016, Nelson et al., 2013) as well as populations with high metabolic disease risk (Ekelund et al., 2016, Swindell et al., 2018). However, most research has been carried out in people of European ancestry who have a lower metabolic disease incidence, including CVD and T2D, compared to many other ethnic groups (Ekelund et al., 2007, Ekelund et al., 2009, Hamasaki et al., 2015, Swindell et al., 2018, Henson et al., 2013). Considering women of Pacific descent are at increased risk of obesity, T2D and CVD compared with women of European descent (Ministry of Health New Zealand, 2021, Sundborn et al., 2008), and that increasing total PA is associated with metabolic health, then encouraging movement

of all intensities, rather than focusing solely on MVPA may be a more achievable public health measure for such populations. However, evidence for this is limited.

The aim of this cross-sectional study was to: 1) determine if objectively measured PA was associated with different metabolic health risk and body fat profiles in a population of healthy, normal-weight and obese women, aged 18-45 years; and 2) determine whether these associations differ between ethnic groups (Pacific and NZE). We hypothesise that higher PA would be associated with lower metabolic risk and lower BF%, and that these associations would not differ between ethnic groups.

4.2 Materials and methods

Participants and setting

Healthy Pacific and NZE women in NZ were invited to take part in the cross-sectional study, PRedictors linking Obesity and the gut MIcrobiomE (PROMISE) (Kindleysides et al., 2019). The methodological details of the PROMISE study have been published previously (Kindleysides et al., 2019).

In brief, inclusion criteria included: women aged 18-45 years, post-menarche and pre-menopausal (as defined by regular menstrual cycles over the last year) in self-reported general good health. Participants were also required to self-identify their ethnicity as follows: being of Pacific ethnicity (self-identified), which required having at least one parent of Pacific ethnicity (no minimum time for living in NZ was required), and NZE ethnicity, which required both parents of European descent (NZ or overseas born) and having themselves lived in NZ for ≥5 years. Participants' self-reported height and weight was collected to enable BMI calculation and categorisation. Women who had a BMI within the predefined normal or obese BMI ranges (BMI ≥18.5 to <25.0 kg/m^2 and ≥30.0 kg/m^2, respectively) were invited to participate with the aim of recruiting approximately equal numbers in each BMI/ethnic group. In recognition that people with the same BMI can have substantial heterogeneity of body fat and metabolic disease risk factors (Oliveros et al., 2014, Kramer et al., 2013, Dickey et al., 1998), participants were subsequently classified into two groups based on BF%: low-BF% (<35%) and high-BF% (≥35%). BF% cut-offs were derived from the American Association of Clinical Endocrinologists and American College of Endocrinology guidelines (obesity in women >35%) (Dickey et al., 1998, Oliveros et al., 2014, Jo and Mainous III, 2018). Exclusion criteria were pregnancy or lactation and presence of any diagnosed chronic illness (e.g., T2D and CVD).

Eligible participants attended our research clinic on two occasions, 11-14 days apart. All participants provided written informed consent prior to participation. The study was approved by the Health and Disability Ethics Committee (HDEC, reference: 16/STH/32) and the trial was registered at anzctr.org.au (ACTRN12618000432213).

Measurements, study procedures and data analysis

Demographic information

Standardised face-to-face interviews with a researcher captured demographic and health information e.g., address, personal/household income and medication use. NZ Deprivation Index 2013 (NZDep2013) (Atkinson J, 2014) was derived from their geographical area of residence, which combines census data relating to home ownership, housing, qualifications, income, employment, access to transport, communications, and family structure. This was used as a measure of socioeconomic status, where a NZDep2013 score of one represents the areas "least deprived" and 10 "most deprived".

Physical activity

To objectively measure PA and sleep, participants wore a triaxial w-GT3X accelerometer (Actigraph, Pensacola, FL) (Actigraph LLC, 2016) on their nondominant hip and an Acti-Watch (Micro Motionlogger®) on their non-dominant wrist, which are the standard placements for measuring movement (Ward et al., 2005) and sleep (Ancoli-Israel et al., 2003), respectively. Both devices were set to record an epoch length of one minute. Participants were instructed to wear both devices continuously (24-hour protocol) for the following eight days, except while bathing or participating in water activities such as swimming, thus not contributing towards the overall activity levels measured by the Actigraph. During the monitor wear period, participants completed a daily sleep and PA diary recording: 1) sleep onset; and end times for any sleep, including naps of ≥10 minutes duration; 2) any intentional physical activity (exercise) they engaged in, describing the type (e.g. running, pump class at gym, walking); start time, duration and perceived intensity of the exercise session. Participants were asked to press the Acti-Watch "event" button when they went to bed and turned off their light to sleep, and when they woke up. This was to mark sleep onset and wake time in the memory of the Acti-Watch.

The w-GT3X accelerometer data were processed using Acti-Life® software (version 6.13.3, Actigraph). Data from the first and last days (days of partial wear) of monitoring were excluded from analysis due to incomplete data. Data was focused on PA, using a midnight-midnight 24-

h data format. All accelerometer epochs that occurred during sleep periods were identified using the sleep diaries and autographically confirmed using Acti-Watch data sleep times, recorded, and subsequently removed, leaving only epochs that occurred during waking hours for analysis. To autographically confirm sleep diaries a trained researcher visually inspected participant's individual Acti-Watch download graph (using WatchWare software version 1.94.0.0) concurrently with the sleep diary. If the sleep diary stated the participant was sleeping and either: 1) the participant had pressed the sleep onset/wake button; or 2) the Acti-Watch graph visually indicated the participant was sleeping, this was confirmed as a correct sleep period. If no time was written in the sleep diary, the Acti-Watch time was used.

Non-wear time for the w-GT3X was defined as ≥60 consecutive minutes of zero epoch counts, with allowance for two minutes of counts between zero and 100. Participant's data were considered valid if they wore the accelerometer for ≥12 hours/day (Matthews et al., 2012), on ≥ four days, including one weekend day (Tudor-Locke et al., 2012). All invalid data were removed; the remaining epochs were categorised into time (min/day) spent in the following levels of PA (counts/min, cpm), using widely-used and validated cut-points (Troiano et al., 2008); sedentary behaviour (0 – 99), and light (100 - 2019), moderate (2020-5998) and vigorous intensity PA (≥5999) and MVPA, a composite measure of moderate and vigorous PA (≥2020). The average daily vector magnitude counts per minute (cpm) was calculated as an indicator of total PA volume (i.e., total daily movement).

Anthropometry

Height was measured to the nearest millimetre with a calibrated Harpenden stadiometer. Fasted body weight was measured on calibrated electronic scales (Sauter platform scale E1200, GmbH, Germany) to the nearest 0.01 kg. Hip and waist circumference were measured with a flexible steel tape (Lufkin W600PM) to the nearest 0.1 cm. For height, body weight, waist and hip circumference, two measurements were taken; if the second measurement was not within 1% of the first, a third measurement was taken. The mean value was recorded if two measurements were taken. If three measurements were taken the median value was recorded. Measured weight and height were used to calculate BMI (kg/m^2). Body composition including total body, android, gynoid and visceral fat was assessed using Dual-energy X-ray Absorptiometry (DXA) (Hologic QDR Discovery A, Hologic Inc with APEX V. 3.2 software) by accredited researchers (Australian and NZ Bone Mineral Society clinical densitometry accreditation) (Kindleysides et al., 2019). Following a 10-minute resting period, blood pressure and heart rate were measured in the supine position with a digital blood pressure monitor

(Omron HEM-907, Omron Healthcare Inc). Three consecutive measurements were taken at 1-min intervals; the mean of the second and third measurements was used for analysis (Egan et al., 2010).

Metabolic health risk factors

Between the hours of 7:30 am and 9:00 am, trained phlebotomists drew 30 ml of blood from fasted participants (overnight, 10-15 hours), to obtain serum and plasma for analysis of metabolic markers and endocrine regulators. Ethylenediaminetetraacetic acid (EDTA) vacutainers (Becton Dickinson) were used for whole blood and stored immediately at −80° C before the remainder of the sample was placed on wet ice until centrifugation. The serum vacutainer was left to stand for between 30 and 60 minutes at room temperature (18° C) to clot. For endocrine regulators, an additional plasma sample (Becton Dickinson vacutainer P800 EDTA, aprotinin, and dipeptidyl peptidase IV) was collected. All EDTA vacutainer tubes were centrifuged at 3500 rpm for 15 minutes at 4° C within one hour of sample collection. Aliquots of plasma and serum were transferred into pre-labelled 1.5 mL microcentrifuge tubes (Eppendorf® safe-lock PCR clean tubes, Hamburg, Germany) and cryovials (Cryo.S Greiner Bio-One, GmbH) and stored immediately at −80° C (Kindleysides et al., 2019).

Plasma levels of leptin were analysed (Plant & Food Research Mt Albert, Sandringham, NZ), using the MILLIPLEX MAP Human Metabolic Hormone Magnetic Bead Panel 96-Well Metabolism Multiplex Assay (Millipore, USA, Cat # HMHEMAG-34K). Leptin samples were assayed in duplicate and plates were read using the Bioplex 100 Analyzer System (Bio-Rad). The leptin range was 0.37–66.0 ng/mL [inter-assay CV: 4.1%, intra-assay CV: 3.5%] and measurements had acceptable percentage of values above the limit of detection (98.4%).

Serum levels of insulin were measured using the electrochemiluminescence immunoassay (ECLIA) method (Roche Diagnostics, Mannheim, Germany) using the Cobas e411 analyser (Hitachi High Technologies Corporation, Tokyo, Japan). The inter-assay %CVs for insulin was 0.5%.

Total cholesterol and non-esterified fatty acids (NEFA) and triglycerides (Trig), HDL, low density lipoprotein (LDL), C-Reactive Protein (CRP), glucose and insulin, were measured using a Cobas e411 automatic electronic analyser (Roche, New Zealand), with kits supplied by Roche Diagnostics (Mannheim, Germany). Inter-assay coefficients of variation for total cholesterol, NEFA, Trig, HDL, LDL, CRP, glucose and insulin were 2.0, 3.4, 0.8, 6.1, 0.9, 3.3, 0.7 and 0.5%, respectively. EDTA whole blood was used to measure Glycated haemoglobin

(HbA1c) levels by turbidimetric inhibition immunoassay (Roche Diagnostic, Mannheim, Germany) on a Hitachi c311 autoanalyser (Hitachi High Technologies Corporation, Tokyo, Japan). The inter-assay %CV for HbA1c was 1.0%. Insulin resistance was calculated using HOMA of insulin resistance (HOMA-IR) (HOMA-IR = (fasting plasma insulin (pmol/ml) x fasting blood glucose (mmol/L))/ 22.5) (Wallace et al., 2004).

Statistical analysis

All statistical analyses were performed using IBM SPSS software for Windows version 24.0 (SPSS Inc, Chicago, IL). Normality of data was tested using histograms and Kolmogorov-Smirnov tests. Fasting insulin and CRP were logarithmically transformed (*ln*) to ensure a normal distribution. For descriptive statistical analysis, participants were categorized into two groups based on ethnicity (Pacific or NZE) and two further subgroups based on predetermined BF% criteria (low <35 or high ≥ 35 BF%). Means and standard deviations (SD) were used to summarise all continuous data, except variables that were logarithmically transformed, which were presented as geometric means and geometric standard deviations (GSD). Frequencies (%) were reported for categorical variables. Differences between ethnic and BF% groups were measured with one-way ANOVA, with Bonferroni post hoc correction. Due to NZE women being significantly older ($p<0.05$) and less deprived ($p<0.05$) than Pacific women and considering age and deprivation are negatively associated with metabolic health (Ministry of Health New Zealand, 2021), all further analysis was carried out controlling for age and deprivation index (NZDep2013). Further adjustment for wear time did not materially affect the study outcomes (data not shown).

Separate multiple linear regression models were used to assess the association of MVPA and total PA with body composition and metabolic health markers, with adjustment for age and NZDep2013 as potential confounders. For assessing the independent association of MVPA and total PA with metabolic health markers, further adjustment for BF% group was conducted. Regression coefficients (β) obtained from log-transformed data represent relative differences and were expressed as a ratio (by using e^{β}). For total daily PA we expressed the difference in body composition and metabolic health markers per 100 units increase of daily PA; for MVPA we expressed the difference per 10 units increase. Analyses were conducted separately for NZE and Pacific participants, as well as for both groups combined. For combined (NZE and Pacific) analyses, adjustments for ethnicity were made and an interaction term was included to interpret the interaction between ethnicity and PA (total PA and MVPA).

4.3 Results

Participant characteristics

Analyses were conducted on 275 women (Figure 4.1). Physical activity, anthropometric and adiposity characteristics of participants are presented in Table 4.1. Pacific women were younger (p<0.05) and had higher NZDep2013 scores than NZE women (p<0.05).

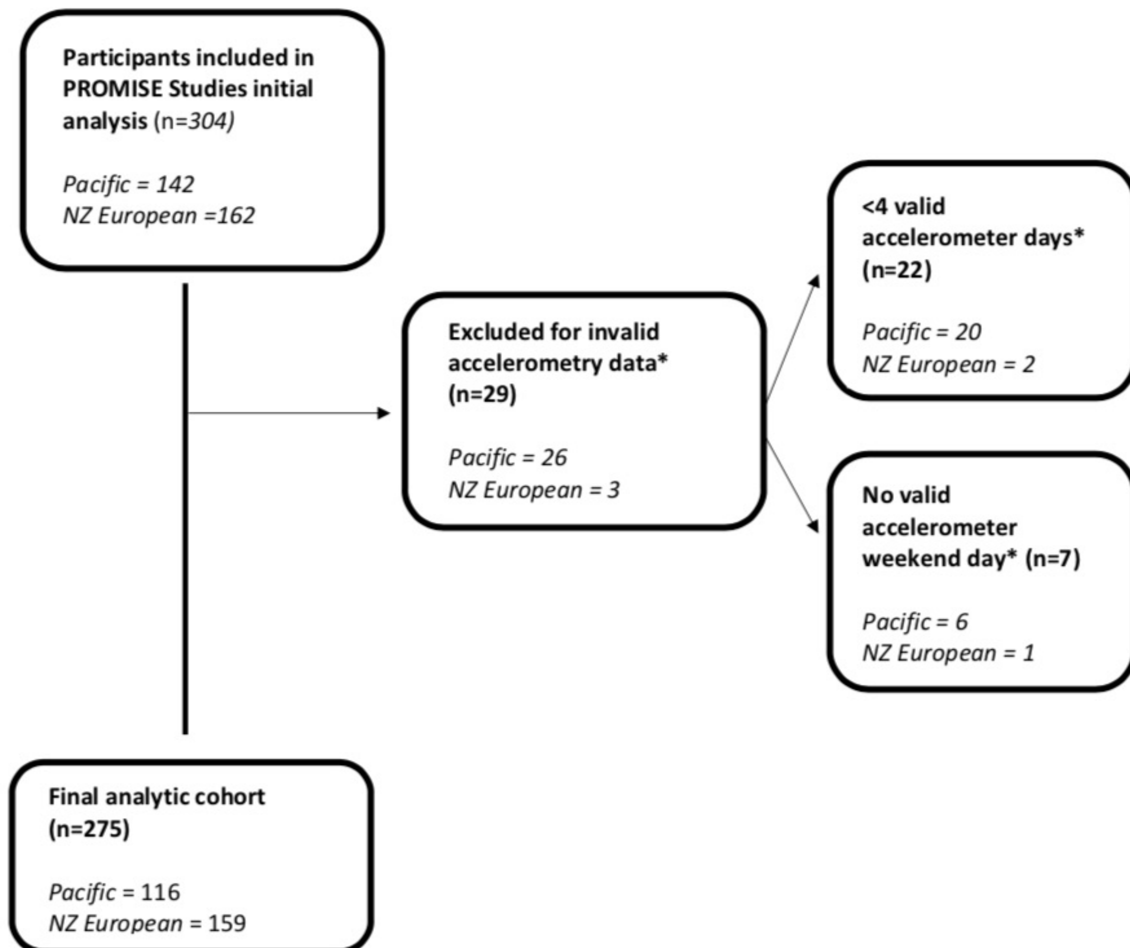

Figure 4.1 Flowchart of participants included in analysis

Physical activity

A weak positive Pearson's correlation was found between MVPA and light intensity PA (LIPA); r=0.178 (p<0.01). Participants achieved a mean of 6 days of valid accelerometer recordings. All four groups' mean waking wear time was >15 hours per day and no significant

difference between groups were found: Pacific high- (15.6 (1.3) hr/day) and low-BF% (15.6 (1.2) hr/day) and NZE high- (15.2 (1.1) hr/day) and low-BF% (15.2 (1.0) hr/day; Table 4.1).

On average participants spent the majority of the waking day in sedentary behaviour (546-596 min/day) (Table 4.1). When comparing means, LIPA was the main intensity of PA in all groups and there was no difference in time spent in LIPA between ethnic or BF% groups. All groups spent less than 3% of the 24-hr day in MVPA, and differences were found between BF% ($p \leq 0.05$) and ethnic ($p \leq 0.05$) groups: Pacific high- (19 min/day) and low-BF% (26 min/day) and NZE high- (31 min/day) and low-BF% (39 min/day; Table 4.1).

Pacific women in the low-BF% group engaged in significantly less total PA when compared to NZE women in the low-BF% group ($p \leq 0.05$), however there was no ethnic difference in total PA between high-BF% groups (Table 4.1).

Body composition

Both Pacific and NZE women's weight, waist and hip circumferences and BMI were significantly higher in the high-BF% group when compared to the low-BF% groups ($p<0.05$; Table 4.1). Pacific women had a higher body weight ($p<0.05$) and had a larger hip ($p<0.05$) and waist ($p<0.05$) circumferences when compared to NZE women. There was no ethnic difference in total or regional BF% in the high BF% group; however, when comparing the low-BF% groups, NZE women had lower total ($p<0.05$), android ($p<0.05$), gynoid ($p<0.05$), visceral ($p<0.05$) and trunk ($p<0.05$) fat percentages.

Biomarkers of metabolic health

Pacific women and women with high-BF% had significantly higher HbA1c and fasting insulin concentrations when compared to NZE women and women with low-BF%. Mean plasma glucose was higher among Pacific women with a low-BF% than for NZE with a low-BF% ($p<0.05$). Glucose was also significantly higher for NZE with a high-BF% when compared to their low-BF% counterparts; Pacific high-BF%, 5.47 mmol/L; and low-BF% 5.31 mmol/L; NZE high-BF%; 5.52 mmol/L; and low-BF%, 5.12 mmol/L (Table 4.1). Among women with a high-BF% NZE women had increased CRP when compared to Pacific women ($p<0.05$). Both NZE and Pacific women's circulating concentrations of triglycerides were significantly higher in the high-BF% group when compared to the low-BF% group and there was no significant ethnic difference. Among high-BF% women, Pacific had significantly lower LDL concentrations compared to NZE. NZE low-BF% women also had significantly lower LDL concentrations compared to NZE high-BF% women. Both Pacific and NZE women's leptin

concentrations were significantly higher in the high-BF% group when compared to the low-BF% groups (Table 4.1), which parallels the BF% measurements described above.

Table 4.1 Characteristics and difference in anthropometry, accelerometery, dietary, socioeconomic and endocrine by ethnicity and body fat % group

Characteristic	Pacific		NZ European	
	<35% body fat Mean (SD)	**≥35% body fat Mean (SD)**	**<35% body fat Mean (SD)**	**≥35% body fat Mean (SD)**
N (%)	61 (22)	55 (20)	85 (31)	74 (27)
BMI (kg·m^{-2})	25.9 (3.9)†^	35.6 (6.1)	22.5 (2.1)†	33.7 (3.8)
Age (years)	25 (7)^	26 (6)^	30 (7)†	33 (7)
Socioeconomic				
NZDep2013	7 (3)^	8 (2)^	4 (2)	5 (2)
Anthropometry				
Weight (kg)	73.7 (11.8)†^	99.7 (18.3)	60.1 (7.6)†	94.4 (13.3)
Height (cm)	169 (6.57)	167 (7.32)	167 (5.61)	167 (7.05)
Waist circumference (cm)	79.6 (8.01)†^	98.5 (13.30)	73.2 (5.75)†	98.0 (9.92)
Hip circumference (cm)	105 (7.06)†^	121.4 (10.84)	98.0 (6.75)†	120.4 (8.75)
Systolic blood pressure	111 (9.3)†	119 (11.4)	113 (10.6)†	120 (13.7)
Heart rate (beats/minute)	69.4 (11.2)	70.5 (11.1)	67.1 (11.4)	74.3 (12.0)
Total body fat (kg)	22.4 (4.99)†^	39.6 (9.80)	17.8 (4.29)†	39.0 (7.68)
Total body fat (%)	30.1 (3.07) †^	39.5 (3.46)	27.8 (4.46)†	41.2 (3.78)
Total lean mass (kg)	51.6 (7.42)†^	59.8 (9.18)^	45.8 (5.26)†	55.3 (7.15)
Android fat mass (kg)	4.70 (1.02) †^	7.30 (1.89)	4.10 (0.74)†	7.22 (1.35)
Android fat mass (%)	29.3 (5.09)†^	41.5 (4.82)	24.8 (5.18)†	41.5 (4.90)
Gynoid fat mass (kg)	12.7 (2.09)†^	16.9 (2.93)	11.0 (1.63)†	16.2 (2.55)
Gynoid fat mass (%)	35.1 (3.39)†	41.0 (3.49)	33.3 (4.20)†	42.5 (4.19)
Visceral fat (%)	27.1 (5.52)†^	39.6 (5.03)	22.2 (5.91)†	39.7 (5.15)
Accelerometery				
Valid days (n)	6 (1)	6 (1)	6 (0)	6 (0)
Participants with only 4 valid days (n)	1	2	2	1
Waking wear time (hr.day^{-1})	15.6 (1.2)	15.6 (1.3)	15.2 (1.0)	15.2 (1.1)
Minimum waking wear time (hr.day^{-1})	13.0	12.2	13.2	12.9
Light PA (min·day^{-1})	314 (76.9)	342 (97.7)	328 (79.4)	321 (85.7)
Sedentary behaviour (min·day^{-1})	596 (85.0)^	575 (98.9)	546 (75.3)	560 (91.5)
MVPA (min·day^{-1})	26.3 (15.6)^	19.1 (15.2)^	39.1 (18.4)†	30.52 (19.1)
Moderate PA (min·day^{-1})	21.5 (13.6)^	18.2 (14.1)^	34.6 (15.3)	29.3 (18.8)
Vigorous PA (min·day^{-1})	1.9 (5.2)^	0.8 (1.7)	4.9 (6.1)†	1.2 (3.0)
Sleep duration (min·day^{-1})	456 (64.5)	431 (62.7)^	477 (49.4)	461 (46.5)
Total daily PA (cpm)	598 (168)^	607 (185)	731 (179)	674 (210)
Carbohydrate metabolism				
Glucose (mmol/L)	5.31 (0.48)^	5.47 (0.51)	5.12 (0.31)†	5.52 (0.46)
HbA1c (mmol/mol)	32.2 (1.99)†^	34.1 (2.97)^	30.4 (2.19)†	31.6 (2.81)
HOMA-IR	21.6 (20.1)†^	39.4 (38.0)^	10.2(4.5)†	22.4 (13.3)
Lipid profile				
Chol (mmol/L)	4.60 (0.72)	4.74 (0.78)^	4.92 (0.86)†	5.40 (1.18)
HDL (mmol/L)	1.58 (0.32)†^	1.40 (0.32)	1.84 (0.37)†	1.50 (0.32)
LDL (mmol/L)	2.83 (0.66)	2.99 (0.73)^	2.89 (0.83)†	3.53 (1.16)

Characteristic	Pacific		NZ European	
	<35% body fat Mean (SD)	**≥35% body fat Mean (SD)**	**<35% body fat Mean (SD)**	**≥35% body fat Mean (SD)**
NEFA (mmol/L)	0.66 (0.32)	0.81 (0.35)	0.67 (0.32)	0.77 (0.38)
Trig (mmol/L)	0.89 (0.34)†	1.25 (0.64)	0.78 (0.28)†	1.20 (0.51)
Endocrine regulators				
Leptin (pg/ml)	9471 (6605)†	23284 (10922)	6037 (4166)†	26852 (13327)
Insulin (pmol/ml)#	73.0 (1.76) †^	122.8 (1.94)^	40.7 (1.51)†	79.8 (1.62)
Inflammation marker				
CRP (mg/L)#	0.49 (2.56)	1.19 (2.72)^	0.74 (2.81)	2.62 (2.52)

Values are presented as Mean ± standard deviation unless otherwise specified; # geometric mean ± geometric standard deviation); † $p< 0.05$ for test comparing body fat groups within ethnic group; ^ $p< 0.05$ for test comparing between ethnic groups within body fat group.
Abbreviations: BMI, Body mass index; NZDep2013, NZDep2013 index of socioeconomic deprivation; PA, physical activity; Light PA, light intensity physical activity; MVPA, moderate to vigorous physical activity; HbA1c, fasting blood glycated haemoglobin; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; CRP, fasting C Reactive Protein; Chol, fasting blood total cholesterol; HDL, fasting High-density lipoprotein; LDL, fasting Low-density lipoprotein; NEFA , fasting non-esterfied fatty acids; Trig, fasting triglycerides; Pacific n=2 with missing deprivation data, NZE n=1 with missing deprivation data.

Multiple linear regression analysis

Multiple linear regression analysis showed a significant albeit small inverse association between total PA, and NZE women's total body, trunk, visceral, android and gynoid fat % (Table 4.2). Every 100 cpm increase in total daily PA was associated with 0.7% lower gynoid fat, 0.8% lower total body, trunk and android and 0.9% lower visceral body fat (Table 4.2). However, we observed no significant association between total PA and any measure of body composition in Pacific women. When investigating time spent in MVPA, we observed a significant association with lower total, trunk and gynoid BF% in both NZE and Pacific women, with the associated lower BF% being of the same magnitude in both ethnic groups (Table 4.2). Every additional 10 minutes spent in MVPA was associated with 0.9% lower total and trunk fat and 0.7% lower gynoid fat. In addition, every 10-minute increase in MVPA was inversely associated with android (0.9%) and visceral (1.0%) BF% in NZE women only ($p \leq 0.05$).

Table 4.2 Association between increasing total physical activity (cpm) and MVPA (min/day) and body composition

Body region	**Pacific n= 114**		**NZ European n= 158**		**All participants ≠ n= 272**	
	Total daily PA, Difference (95% CI) per 100 units increase	MVPA, Difference (95% CI) per 10 units increase	Total daily PA, Difference (95% CI) per 100 units increase	MVPA, Difference (95% CI) per 10 units increase	Total daily PA, Difference (95% CI) per 100 units increase	MVPA, Difference (95% CI) per 10 units increase
Trunk fat %	-0.5 (-1.2, 0.3)	-0.95 (-1.80, -0.10)*	-0.8 (-1.5, -0.1)*	-0.91 (-1.63, -0.19)*	-0.7 (-1.2, -0.2)*	-0.95 (-1.49, -0.40)**
Android fat %	-0.5 (-0.4, 0.3)	-0.90 (-1.84, 0.05)	-0.8 (-1.6, 0.0)*	-0.94 (-1.72, -0.16)*	-0.7 (-1.2, -0.1)*	-0.95 (-1.54, -0.36)**
Gynoid fat %	-0.4 (-0.9, 0.1)	-0.65 (-1.20, -0.10)*	-0.7 (-1.2, -0.2)**	-0.65 (-1.15, -0.16)**	-0.6 (-0.9, -0.2)**	-0.67 (-1.04, -0.31)***
Visceral fat %	-0.5 (-0.4, 0.3)	-0.91 (-1.90, 0.07)	-0.9 (-1.7, 0.0)*	-1.00 (-1.83, -0.17)*	-0.7 (-1.3, -0.1)*	-0.99 (-1.62, -0.37)**
Total body fat %	-0.4 (-0.0, 0.2)	-0.89 (-1.56, -0.21)*	-0.8 (-1.4, -0.2)**	-0.85 (-1.47, -0.23)**	-0.6 (-1.1, 0.2)**	-0.88 (-1.33, -0.42)***
Waist circumference (cm)	-0.5 (-1.9, 1.0)	-1.37 (-3.05, 0.31)	-0.6 (-1.8, 0.5)	-1.39 (-2.52, -0.27)*	-0.7 (-1.6, 0.2)	-1.58 (-2.46, -0.70)***
BMI ($kg.m^{-2}$)	0.1 (-0.7, 0.8)	-0.46 (-1.30, 0.37)	-0.3 (-0.8, 0.2)	-0.53 (-1.03, -0.40)*	-0.3 (-0.7, 0.1)	-0.64 (-1.05, -0.23)**

All models adjusted for age and NZDep2013; ≠ model further adjusted for ethnicity; *p<0.05, **p< 0.01, ***p<0.001; Regression coefficients represent the change in the outcome per 100 cpm change in total daily PA and 10-minute change in MVPA. NZ, New Zealand; PA, physical activity; MVPA, moderate to vigorous physical activity.

Among Pacific women, significant inverse associations were found between total PA and fasting plasma insulin. Every 100 cpm increase in total PA was associated with a 9% lower fasting plasma insulin; this association was not significant in NZE women (Table 4.3). For both Pacific and NZE women, every 10-minute increase in MVPA was associated with 8% lower fasting plasma insulin ($p \leq 0.05$) (Table 4.3).

Among NZE women every 100 cpm increase in total PA and 10-minute increase in MVPA was significantly associated with 9% and 10% lower ($p \leq 0.05$) fasting CRP concentration, respectively (Table 4.3); no significant association was observed in Pacific women.

A significant inverse association was also found between increasing total PA and heart rate for NZE women: every 100 cpm increase in daily PA was associated with a lower resting heart rate of 1.4 beats per minute ($p<0.05$). Further, every additional 10 minutes spent in MVPA was significantly associated with a lower heart rate in Pacific and NZE women (1.8 and 1.2 beats/minute, respectively) and significantly lower HOMA-IR (0.36) among NZE women. Small significant positive associations were also observed with increasing total PA and Pacific women's systolic blood pressure and NZE women's HbA1c concentrations ($p<0.05$; Table 4.3).

Table 4.3 Association between total daily PA (cpm) and MVPA (min/day) and metabolic health markers

Biomarker	Pacific N=114		NZ European n= 158		All participants ≠ n= 272	
Endocrine	Total daily PA, Difference (95% CI) per 100 units increase	MVPA, Difference (95% CI) per 10 units increase	Total daily PA, Difference (95% CI) per 100 units increase	MVPA, Difference (95% CI) per 10 units increase	Total daily PA, Difference (95% CI) per 100 units increase	MVPA, Difference (95% CI) per 10 units increase
HbA1c (mmol.mol)	-0.1 (-0.3, 0.2)	0.1 (-0.2, 0.4)	0.2 (0.0, 0.4)*	-0.1 (-0.3, 0.1)	0.1 (0.0, 0.1)	0.0 (-0.2, 0.2)
Glucose (mmol.L)	0.0 (-0.1, 0.0)	-0.01 (-0.07, 0.05)	0.0 (0.0, 0.0)	-0.03 (-0.06, 0.00)	0.0 (0.0, 0.0)	-0.02 (-0.05, 0.01)
HOMA-IR	-2.8 (-5.9, 0.4)	-2.6 (-6.3, 1.0)	0.2 (-0.6, 0.9)	-0.9 (-1.7, -0.1)*	-0.9 (-2.2, 0.4)	-1.5 (-2.9, -0.03)*
Leptin (pg.ml)	-729 (-1711, 251)	-989 (-2090, 112)	-26 (-826, 774)	31 (-794, 857)	-304 (-917, 310)	-267 (-920, 387)
Chol (mmol.L)	0.0 (-0.1, 0.1)	0.05 (-0.03, 0.14)	0.0 (-0.1, 0.1)	0.03 (-0.03, 0.09)	0.0 (0.0, 0.1)	0.01 (-0.05, 0.07)
NEFA (mmol.L)	0.0 (0.0, 0.1)	-0.01 (-0.08, 0.05)	0.0 (0.0, 0.0)	-0.03 (-0.06, 0.00)	0.0 (0.0, 0.0)	0.02 (0.00, 0.05)
Trig (mmol.L)	0.0 (-0.1, 0.0)	-0.01 (-0.08, 0.05)	0.0 (0.0, 0.0)	-0.03 (-0.06, 0.00)	0.0 (-0.1, 0.0)	-0.03 (-0.06, 0.01)
HDL (mmol.L)	0.0 (0.0, 0.1)	0.03 (0.00, 0.07)	0.0 (0.0, 0.0)	0.04 (0.02, 0.06)***	0.0 (0.0, 0.0)	0.03 (0.01, 0.06)**
LDL (mmol.L)	0.0 (-0.1, 0.1)	0.03 (-0.06, 0.11)	0.0 (0.1, 0.1)	0.01 (-0.05, 0.06)	0.0 (0.0, 0.1)	0.00 (-0.06, 0.06)
Anthropometry						
Weight (Kg)	0.4 (-1.2, 2.0)	0.49 (-1.49, 2.38)	0.3 (-0.6, 1.2)	-0.12 (-1.03, 0.78)	0.3 (-0.5, 1.1)	0.12 (-0.77, 1.01)
Waist circumference (cm)	-0.5 (-1.6, 0.6)	-0.50 (-1.79, 0.79)	0.4 (-0.2, 1.1)	-0.14 (-0.81, 0.52)	0.1 (-0.5, 0.6)	-0.22 (-0.85, 0.41)
Hip circumference (cm)	0.1 (-0.9, 1.0)	0.22 (-0.90, 1.33)	0.1 (-0.6, 0.7)	-0.04 (-0.70, 0.62)	0.0 (-0.5, 0.6)	0.09 (-0.49, 0.66)
Systolic blood pressure	1.1 (0.1, 2.2)*	0.45 (-0.79, 1.70)	0.1 (-1.0, 1.1)	-0.30 (-1.34, 0.74)	0.4 (-0.3, 1.1)	0.00 (-0.79, 0.79)
Diastolic blood pressure	0.5 (-0.3, 1.4)	-0.17 (-1.20, 0.86)	-0.5 (-1.2, 0.3)	-0.045 (-0.122, 0.031)	-0.2 (-0.7, 0.4)	-0.29 (-0.90, 0.33)
Heart rate (bpm)	-1.1 (-2.3, -0.2)	-1.83 (-3.19, -0.48)**	-1.4 (-2.4, 0.4)**	-1.21 (-2.26, -0.17)*	-1.2 (-2.0, -0.4)**	-0.14 (-2.26, -0.63)**
Endocrine	Total daily PA, Ratio (95% CI) per 100 units increase	MVPA, Ratio (95% CI) per 10 units increase	Total daily PA, Ratio (95% CI) per 100 units increase	MVPA, Ratio (95% CI) per 10 units increase	Total daily PA, Ratio (95% CI) per 100 units increase	MVPA, Ratio (95% CI) per 10 units increase
Insulin† (pmol/ml)	0.91 (0.91, 1.00)*	0.92 (0.85, 0.99)*	1.00 (1.00, 1.00)	0.92 (0.90, 0.96)***	1.00 (0.91, 1.00)*	0.95 (0.91, 0.98)**
CRP† (mg.L)	0.91 (0.82, 1.00)	0.96 (0.85, 1.10)	0.91 (0.82, 1.00)*	0.90 (0.83, 0.98)*	0.91 (0.82, 1.00)**	0.92 (0.87, 0.99)*

NZE, New Zealand European; PA, physical activity; MVPA, moderate to vigorous physical activity; HbA1c, fasting blood glycated hemoglobin; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; CRP, fasting C Reactive Protein; Chol, fasting blood total cholesterol; HDL, fasting High-density lipoprotein; LDL, fasting Low-density lipoprotein; NEFA, fasting non-esterfied fatty acids; Trig, fasting triglycerides. All models adjusted for body fat % group, age and NZDep2013; ≠further adjusted for ethnicity; *$p<0.05$, **$p< 0.01$, ***$p<0.001$; † data has been log transformed (*ln*). Regression coefficients represent the change in the outcome per 100 cpm change in total daily PA and 10-minute change in MVPA

4.4 Discussion

Results from this study indicate, that in a healthy population, increasing PA, in particular MVPA, is associated with improved BF% and blood glucose metabolism and lower heart rate for Pacific and NZE women, despite different metabolic disease risk, body fat profiles and deprivation level.

Physical activity and body composition

In this study, increased MVPA was associated with lower total body, trunk and gynoid fat% in both Pacific and NZE women. A large amount of cross-sectional, longitudinal and intervention research has been carried out investigating the associations between MVPA and body composition. However, systematic reviews and meta-analysis collating this evidence report equivocal results (Baillot et al., 2014, Wilks et al., 2011, Fogelholm and Kukkonen-Harjula, 2000, Conn et al., 2014, Hebden et al., 2012, Franz et al., 2007), which may be due to differences in the experimental design and methodology making studies difficult to compare: Baillot et al. only investigated individuals with obesity (Baillot et al., 2014), some only considered weight gain as an outcome (Fogelholm and Kukkonen-Harjula, 2000, Hebden et al., 2012), others assessed body composition through BMI (Gibbs et al., 2017, Wagner et al., 2001, Britton et al., 2012, Ekelund et al., 2017) and waist circumference (Gibbs et al., 2017, Wagner et al., 2001), whilst some investigated more detailed measures of body composition such as BF% (Wilks et al., 2011, Baillot et al., 2014, O'Brien, 2018). In a similar population of NZ European and Pacific women, who engaged in similar amounts of MVPA to the current study (35 and 22 min/day respectively), O'Brien et al. (2018), found no association between increasing MVPA and any BF% region they measured using DXA (gynoid, android, whole BF%) in either Pacific or NZE women (O'Brien, 2018). In this study, higher total PA was associated with significantly lower total, trunk, android, gynoid and visceral BF% amongst NZE women. Although these same trends were observed amongst Pacific women, results were not significant. Research investigating associations between total PA and BF% is scarce, thus it is difficult to compare to previous research. None-the-less, Wolff-Hughes (2015) found similar results to our NZE women, reporting significant inverse associations with total PA and triceps and subscapular skinfold measurements.

A notable finding was the significant inverse association between increasing total PA and MVPA and visceral and android fat % amongst NZE, but not Pacific women. Pacific and NZE women had similar total body fat mass; however, low-BF% Pacific women had significantly higher visceral adiposity when compared to their NZE counterparts. This finding is important,

as higher visceral adiposity has long been associated with increased risk of non-communicable diseases and inflammation (Zhang et al., 2015, Fontana et al., 2007, Kang et al., 2015, Fox et al., 2007, Stępień et al., 2014), and thus may contribute towards Pacific women's increased susceptibility to metabolic diseases (Swindell et al., 2018, Healy et al., 2008, Henson et al., 2013). Pacific women may therefore require higher levels of total PA and/or MVPA to experience a similar decrease in total and regional BF% as observed in NZE women.

Physical activity and biomarkers of metabolic health

High-BF% was associated with significantly higher CRP concentrations (Table 4.1). This is suggestive of the influence of adipose tissue on the pro-inflammatory state (Fontana et al., 2007). It is interesting to note that Pacific women had significantly lower CRP levels compared to NZE women; this is despite having a similar total BF%. Also, Pacific women in this study had fasting plasma insulin concentrations almost two-fold higher than their NZE counterparts. Our results are consistent with other research comparing insulin concentrations and CRP levels of NZE and Pacific women (McAuley et al., 2002, O'Brien, 2018, Murphy et al., 2019, Whitford, 2017). It is important to note that participants in this study were "healthy" and had not been diagnosed with a chronic disease. Mean CRP for both ethnic groups were <5.0 mg/L, suggesting no presence of clinical inflammation. Further, the increased insulin concentrations observed in Pacific women of the present study did not appear to have elicited a chronic pathophysiological response (i.e. all participants had normal HbA1c level below 41 mmol/mol (Labtests, 2021)). Further, it is well documented that sustained increased levels of insulin may indicate a reduced degree of insulin sensitivity; therefore, the observed profile may be indicative of the preliminary stages of insulin hypersecretion and early signs of reduced insulin sensitivity among these Pacific women. Previous research has shown that, when compared to NZE, South Pacific people (NZ Māori) had lower insulin sensitivity (measured using euglycemic insulin clamp) at an equivalent level of BMI (McAuley et al., 2002). However, this population consisted of NZ Māori (despite being described as South Pacific people), and metabolic risk (or profiles) is different between NZ Māori and other Polynesian groups, such as participants in our study (Merriman and Wilcox, 2018). Whilst the mechanisms underlying this observation cannot be determined from this study, ethnic differences in the low BF% group's fasting insulin concentrations may in part be explained by their higher visceral adiposity; this, however, does not explain the ethnic differences in high BF% groups fasting insulin concentrations. However, two important findings of this study may also help explain these ethnic differences. Firstly, Pacific women in the high BF% group engaged in significantly

less MVPA when compared to the NZE group, and secondly, MVPA was significantly associated with lower insulin in both ethnic groups. This emphasises the importance of encouraging all women to engage in PA, including MVPA.

Although the positive associations found between increasing total PA and Pacific women's systolic blood pressure and NZE women's HbA1c concentrations should be investigated further, they are unlikely to be clinically relevant and may be a statistical anomaly. For example, if Pacific women in this study were to increase their total PA by as much as 50%, they would only see a 3.3 mmHg increase in their systolic blood pressure (mean total daily PA; 598 and 607 cpm in the low- and high-BF% groups respectively). Further, for NZE participant's HbA1c to increase by as little as 1.0 mmol/mol they would need to increase their total daily PA by 500 cpm, which equates to an increase of at least 75%.

Within our population, there was an inverse association between total PA and key markers of metabolic health (insulin, CRP and heart rate). Other research in European populations has also reported increased total PA to be significantly positively associated with metabolic health including fasting insulin, HOMA_IR, and CRP (Swindell et al., 2018, Ekelund et al., 2007), thus it is promising to also see metabolic benefits of total PA in other ethnic groups (i.e. Pacific) with higher metabolic disease risk. It is particularly interesting to observe a significant inverse association between total PA and fasting insulin concentrations among Pacific, but not NZE women. This association may be explained by fasting plasma insulin concentrations among Pacific women being two-fold higher than those of the NZE women, hence the capacity for change was greater among Pacific women. This is an important finding considering Pacific women's total PA was significantly lower than NZE women's and it was not associated with body composition. Similarly, it was interesting to find no association between Pacific women's CRP and total PA or MVPA, whereas significant negative associations among NZE women were observed. CRP is positively associated with visceral BF% (Sanip et al., 2013), which may explain the finding that increasing total PA and MVPA was inversely associated with visceral fat% amongst NZE women, but not in our Pacific population. Others report high levels of exercise and energy restriction leading to significant weight loss including reduction in visceral fat mass can improve cardiometabolic profile through inflammation-related biomarkers including CRP (Sarin et al., 2019).

Considering there is growing concern for the impact of sedentary behaviour on metabolic health, independent of PA, it is encouraging to see these associations between PA, adiposity and metabolic health in a sedentary population. Women in the current study were sedentary for

a substantial portion of their waking day (>9 hours/day). Comparable to our population, Canadian adults spend about 9-10 hours/day in sedentary behaviour (Prince et al., 2020). However, other populations are considerably less sedentary (Matthews et al., 2008, Beale et al., 2020). Also using accelerometery data, Beale et al. (2020) investigated a similar population of NZ women to the current study, where participants spent an average of 7 hours 42 min/day in SB (Beale et al., 2020). In comparison, data from the U.S. National Health and Nutrition Examination Survey (NHANES) indicate that approximately 55% of children's and adult's awake time (7.7 hours·d−1) is spent being sedentary (Matthews et al., 2008), which is over one hour less than women in the current study.

Pacific women in this study spent less time in PA when compared to NZE women, which is consistent with previous research (O'Brien, 2018, O'Brien et al., 2019). O'Brien et al. (2018) also investigated Pacific and NZE women's objectively measured PA patterns and found participants engaged in similar amounts of MVPA to the current study (Pacific; 22 and NZE; 35 min/day) (O'Brien, 2018). The NZE women's mean moderate PA levels fell within the recommended 150-300 min/week (low-BF% 240 and high-BF% 205 min/week) whereas only the Pacific low BF% group met this guideline (low-BF% 150 and high-BF% 128 min/week). This may help to explain the stronger inverse association between MVPA, total PA and adiposity and the majority of markers of metabolic health (HOMA_IR, CRP, HDL) observed in NZE women. However, it is important to note that this result may also be due to greater variance of PA measures (total daily PA and MVPA) observed in NZE women compared to Pacific women, thus resulting in more statistical power for the analyses involving NZE women.

Research has shown that a large portion of the association between socioeconomic position and obesity is mediated through health behaviours such as diet and physical activity (Shaikh et al., 2015, Pampel et al., 2010, Foster et al., 2018). The more deprived, the more vulnerable people are to the obesogenic environment, access to food and PA which is limited by cost, time and geographical locations (Exeter et al., 2017). Areas of high deprivation may have smaller tax income to fund recreational PA facilities, whilst simultaneously having higher crime rates, a barrier to engaging in PA in public spaces (Exeter et al., 2017).

Our results may suggest that PA is more effective at improving insulin and CRP in one ethnic group when compared to another but may depend on baseline insulin concentrations and CRP levels. Future studies could address pathways of insulin secretion, chronic systemic inflammation or the gut microbiome (Mailing et al., 2019), all of which have been implicated as pathways to obesity and increased metabolic disease risk.

Strengths and limitations

The objective measurement of PA is a strength to this study, as is the 24-hour accelerometer wear protocol which allowed for objective measurement of sleep onset and wake times. Consequently, we could accurately remove all sleep data from the PA analysis without relying on algorithms to predict this data. However, we acknowledge wearing an accelerometer may in some individuals result in an increase of PA, particularly on the first day of monitoring (Baumann et al., 2018). To reduce the potential of overestimating average PA levels we have excluded data from the first day of monitoring; however, we cannot exclude the possibility that wearing an accelerometer may have also affected PA in subsequent days. Nonetheless, we would not expect this to be different between low and high BF% groups and Pacific and NZE women. Therefore, we do not believe that any increases in PA due to wearing an accelerometer would have resulted in significant bias.

The 24-hour day is finite, therefore the time spent in all movement behaviours is co-dependent (sleep, sedentary behaviour, light intensity PA and MVPA); associations we observed between PA, body composition and metabolic markers of health may have reflected the highly sedentary nature of this population. Future studies should consider using statistically advanced analytical methods such as compositional analysis that account for this (Chastin et al., 2015).

There is no formal agreement on defining obesity in terms of BF% (Oliveros et al., 2014). However, a wide variety of BF% cut-off points have been used for women, varying between 30 to 37% (Oliveros et al., 2014). The chosen cut-point of for categorising high vs low BF% (35%) was confirmed after carrying out analysis within our population comparing BF% with BMI. In particular, scatter plots, graphing BMI vs BF% with a line of best fit suggested using 35% BF as our cut-point. Using this cut point only five participants with a BMI <25 kg/m^2 were in the >35% BF group, suggesting potential misclassification in terms of BMI was minor. Further, cross-sectional designs are susceptible to reverse causality. As PA behaviour may change as a consequence of obesity, caution must be taken when interpreting these results. Due to the nature of the PROMISE study design, the adjustment for BF% group was necessary, however, body fat% could be part of the pathway between PA and poor metabolic health. Therefore, it is important to note that it is possible, adjusting for BF% group could have subjected the analyses to over adjustment. It is also possible that PA behaviours cluster with other unhealthy activities such as dietary or sleep patterns or increased sedentary behaviour.

4.5 Conclusion

Objectively measured PA was associated with lower metabolic risk markers in both Pacific and NZE women. Fasting insulin concentrations differed between ethnic and BF% groups with an increased risk of hyperinsulinemia in Pacific women and women with obesity. Increases in total daily PA and MVPA were associated with lower fasting plasma insulin and CRP as well as heart rate, thus indicating a lower metabolic disease risk. These findings confirm the importance of PA but suggest the potential health benefit may differ between ethnic groups. Importantly, increased total PA appears to have a greater impact on Pacific women's fasting insulin concentrations when compared to NZE. Considering Pacific women are a high metabolic disease risk population and 9.5% are diagnosed with diabetes (Ministry of Health New Zealand, 2021), promotion of PA in this population should remain a priority.

4.6 References

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Chapter 5: Objectively measured sedentary behaviour and metabolic health in Pacific and New Zealand European women.

Abstract

Aims: To assess: 1) sedentary behaviour (SB) in Pacific and New Zealand European (NZE) women; 2) associations between SB and metabolic risk and body composition; and 3) whether associations differ between ethnic groups.

Methods: Pacific (n=119) and NZE (n=159) women, aged 18-45 years were recruited according to self-reported normal or obese body mass index (BMI). SB was assessed using eight-day accelerometery. Fasting blood was assessed for biomarkers of metabolic health. Body composition was assessed using BMI, waist and hip circumference, and BF% (whole body dual-energy x-ray absorptiometry). Body fat % was used to stratify women as low (<35%) or high (≥35%) BF%. Linear regression assessed associations between SB, body composition and metabolic markers.

Results: Low-BF% Pacific women were more sedentary than all other women ($p<0.05$): Pacific low- 10.37 and high-BF% 9.93 and NZE low- 9.69 and high-BF% 9.96 min/day. NZE women with high-BF% had a higher ($p<0.05$) weighted median sedentary bout length (20.07 min) compared to NZE low-BF% (17.81 min) and Pacific high-BF% women (18.32 min). Among NZE women, every one-hour increase in sedentary time was associated with 0.8% higher gynoid fat ($p<0.05$), and weighted median sedentary bout length was associated with BF% (gynoid fat 0.3, total body 0.4, trunk 0.4, android 0.4 and visceral fat 0.4 ($p<0.05$)). No associations between SB and body composition were found among Pacific women. Among NZE but not Pacific women, every one-hour increase in sedentary time was associated with a 14% higher C-reactive protein ($p<0.05$).

Conclusion: Our findings indicate breaking-up prolonged SB may assist in achieving healthier body composition. Also, inflammation (CRP) was associated with SB, this association may impact metabolic disease risk, especially NZE, when compared to Pacific women.

5.1 Introduction

Obesity is increasingly prevalent and has been identified as one of the key risk factors for cardiovascular disease (CVD), type 2 diabetes (T2D), and some cancers (Guh et al., 2009). In developed countries, obesity is predominantly observed in socioeconomically disadvantaged populations (Zhang and Wang, 2004) and among women (NCD Risk Factor Collaboration, 2016, GBD 2015 Obesity Collaborators, 2017). This is of particular concern in New Zealand (NZ), which ranks among the countries with the highest obesity rates in the world (OECD, 2017) and is disproportionally prevalent in some minority populations, including NZ Māori and Pacific peoples. In particular, 62% of NZ Pacific women have a BMI ≥30 kg/m^2 (or International Obesity Task Force equivalent for 15-17 years) compared to 30% of NZ European (NZE)/Other women (who reported "New Zealander" or another (non-Māori, non-Pacific, non-Asian) ethnic affiliation) (Ministry of Health New Zealand, 2021). Ethnic differences are also observed in the prevalence and risk of chronic obesity-related conditions (Bhopal et al., 1999, Volgman et al., 2018). Specifically, Pacific women are at increased risk of poor cardiovascular health and T2D with the prevalence of diabetes being 2.0 times higher among Pacific compared to NZE women (Ministry of Health New Zealand, 2021). The reasons for this are unclear, but may be linked to genetic variance or social/environmental determinants (Swinburn et al., 2011).

Sedentary behaviour (SB), defined as low energy expenditure (≤ 1.5 metabolic equivalents (METs)) in a sitting or reclining posture during waking times, has been linked to obesity and poor metabolic health (Tremblay et al., 2017, Proper et al., 2007). In particular, SB adversely impacts on general metabolic health (Chastin et al., 2015, Brocklebank et al., 2015), diabetes, cardiovascular disease, some cancers and all-cause mortality (Wilmot et al., 2012) (Ekelund et al., 2016) (De Rezende et al., 2014, Lynch et al., 2018). A systematic review and meta-analysis by Wilmot et al. (2012) reported that higher levels of SB were associated with an increase in the relative risk of diabetes (112%), cardiovascular disease (147%), cardiovascular mortality (90%) and all-cause mortality (49%) (Wilmot et al., 2012). It has been suggested that not only the overall quantity of SB is detrimental to metabolic health, but also the pattern in which it is accumulated. A less prolonged sedentary accumulation pattern (i.e., more regular breaks, shorter sedentary bouts) has been associated with lower body mass index (BMI) (Chastin et al., 2015), improvements in postprandial glucose metabolism (Saunders et al., 2018), and all-cause mortality (Diaz et al., 2017). However, findings have been inconsistent with some studies

showing no association between breaks in SB or sedentary bout duration and all-cause mortality (Jefferis et al., 2019).

Part of the World Health Organisation (WHO) strategy for reducing overweight and obesity is to reduce SB (World Health Organisation, 2019) and their guidelines now include a recommendation that “adults should limit the amount of time spent being sedentary” (World Health Organisation, 2020). WHO recommendations stop short of recommending a time limit on SB, reflecting the gap of evidence in this area. PA guidelines for several countries, including Australia, Canada, America and NZ now include recommendations for SB (Brown et al., 2013) (Ross et al., 2020) with Canada the only country to include a recommended upper limit for sedentary time, specifying eight hours or less as a target (Ross et al., 2020). A key difference being Canada focused on research promoting movement over a 24-hour period, integrating PA, SB and sleep (Ross et al., 2020), whereas the new WHO guidelines only focus on MVPA and SB (World Health Organisation, 2020).

Minority groups, including indigenous populations in Europe and immigrants from low and middle income countries had higher levels of SB (Langøien et al., 2017); however, evidence of associations between SB and metabolic health across ethnic groups is scarce (Nagy et al., 2019, Brodersen et al., 2007). Therefore, an improved understanding of patterns of SB (total time, bouts and breaks) in different ethnic groups and the relationship with body composition and metabolic health, could help to inform more specific (and effective) public health guidelines and clinical treatment options. Recently, researchers have called for future research on SB to focus, on obese/overweight populations as well as ethnic minorities (Schmid et al., 2018, Katzmarzyk et al., 2019).

Therefore, the aim of this study was to: describe patterns of SB among a population of healthy, normal-weight and obese, Pacific and NZE women, aged 18-45 years; 2) To determine if objectively measured SB was associated with different metabolic risk and body composition; and 3) whether these associations differ between ethnic groups (Pacific and NZE women).

5.2 Materials and methods

Detailed methods of the wider PRedictors linking Obesity and the gut MIcrobiomE (PROMIsE) study have been published previously (Kindleysides et al., 2019). Inclusion criteria included: women aged 18-45 years, post-menarche and pre-menopausal (as defined by regular menstrual cycles over the last year), in general good health (self-reported). Participants were also required to self-identify their ethnicity as follows: Pacific ethnicity, which required having at least one parent of Pacific ethnicity (no minimum time for living in NZ was required), and

NZE ethnicity, which required both parents to be of European descent (NZ or overseas born) and having themselves lived in NZ for ≥5 years. Participant's self-reported height and weight were obtained to determine BMI for recruitment purposes. Women who had a BMI within the predefined normal or obese BMI ranges (BMI ≥18.5 to <25.0 kg/m^2 and ≥30.0 kg/m^2, respectively) were invited to participate. In recognition that people with the same BMI can have substantial heterogeneity of body fat and metabolic disease risk factors (Oliveros et al., 2014, Kramer et al., 2013, Dickey et al., 1998), participants were subsequently classified into two groups based on body fat percentage (BF%): low-BF% (<35%) and high-BF% (≥35%). BF% cut-offs were derived from the Obesity Medicine Association guidelines (obesity in women >35%) (Fitch and Bays, 2022). Exclusion criteria were pregnancy or lactation and presence of any diagnosed chronic illness (e.g., T2D and CVD).

Eligible participants attended the research clinic on two occasions, 11-14 days apart. Prior to participation, all participants provided written informed consent. The Health and Disability Ethics Committees Ethics committee (HDEC, reference: 16/STH/32) approved the study.

Demographic information

To obtain demographic and health information, a face-to-face questionnaire was administered. NZ Deprivation Index 2013 (NZDep2013) was used as a proxy for assessing socioeconomic status (Atkinson J, 2014). NZDep2013 is derived from geographical area of residence, combining census data relating to home ownership, housing, qualifications, income, employment, access to transport, communications, and family structure. A NZDep2013 score of one represents the areas "least deprived" and 10 "most deprived".

Sedentary behaviour, physical activity, sleep and diet

Movement behaviour was measured with a w-GT3X tri-axial accelerometer (Actigraph, Pensacola, FL, United States) (Actigraph LLC, 2016) and an Acti-Watch (Micro Motionlogger®). Participants were fitted with accelerometers at clinic visit one and they were worn for the following eight days. The w-GT3X was worn on the nondominant hip and the Acti-Watch on the non-dominant wrist (Ward et al., 2005) (Ancoli-Israel et al., 2003). Both devices were set to record epoch length of one minute. Participants wore both devices continuously (24-hour protocol), and were instructed to remove the accelerometer only for water-based activities (e.g., bathing or swimming). During this period, participants completed a daily PA and sleep diary recording: any time the participant removed and put back on accelerometers; what they were doing in the time they were not wearing the accelerometers (e.g swimming, bathing); sleep onset; and end times for any sleep ≥10 minutes duration. To

record sleep onset and wake time, participants pressed the Acti-Watch "event" button when they went to bed and turned off their light to sleep, and again when they woke up.

Acti-Life® software (version 6.13.3, Actigraph) was used to process the w-GT3X accelerometer data. Data sets were focused on SB using a midnight-midnight 24-h data format. The first and last days of monitoring (days of partial wear) were excluded from the w-GT3X data analysis due to incomplete data. All accelerometer epochs that occurred during sleep periods were identified using the sleep diaries and were confirmed using ActiWatch accelerometer data. Sleep periods were then removed, therefore analysis only included epochs that occurred during waking hours. A trained researcher visually inspected participant's individual ActiWatch accelerometer download graphs (using WatchWare software version 1.94.0.0) simultaneously with their sleep diary. If the sleep diary stated the participant was sleeping and either: 1) the participant had pressed the sleep onset/wake button; or 2) the ActiWatch graph visually indicated the participant was sleeping, this was confirmed as a correct sleep period. The ActiWatch time was used if no time was written in the sleep diary.

Non-wear time for the wGT3X was defined as ≥ 60 consecutive minutes of zero epoch counts, with allowance for two minutes of counts between zero and 100. Participant's data were considered valid if the accelerometer was worn for ≥12 hours/day (Matthews et al., 2012), on ≥ four days, including one weekend day (Tudor-Locke et al., 2012). All data that did not meet this criteria were removed. Six different movement behaviour modes were calculated from valid data; mean time (min/day) spent in SB (0 – 99 counts/min), light intensity physical activity (LPA) (100 - 2019 counts/min) and moderate to vigorous intensity physical activity (MVPA) (≥2020 counts/min) (Troiano et al., 2008), weighted median sedentary bout length (≥10 minutes), maximum sedentary bout length, and fragmentation index. Weighted median sedentary bout length is the length of the sedentary bout corresponding to 50% of total daily accumulated sedentary time. For example, if a participant recorded six hours of sedentary time, after ordering bouts cumulatively from smallest to largest, the weighted median sedentary bout length represented the length of the bout that contained the three-hour time point. The higher the weighted median bout length, the less interrupted the SB. The fragmentation index (an index of breaks in sitting time), is the ratio of the number of sedentary bouts (≥10 minutes) divided by total sedentary time. A higher fragmentation index indicates more interrupted SB. Vector magnitude mean activity count per minute (cpm), during valid wear time was calculated as an indicator of total PA volume (i.e. total daily movement). To assess sleep quality, participants completed the validated, Pittsburgh Sleep Quality Index (PSQI) (Buysse et al.,

1989), as an online, self-administered, forced-choice sleep quality questionnaire, hosted by Survey Monkey © (SurveyMonkey Inc, 2017). Total daily energy intake was calculated using data from a 5-day estimated food record and analysed through FoodWorks dietary analysis software (FoodWorks Professional 9; Xyris Software, Australia; New Zealand Food Composition Database).

Anthropometry

All anthropometric measurements were conducted using the International Society for the Advancement of Kinanthropometry (ISAK) protocol (Stewart A, 2011). All research staff conducting these measurements were trained according to ISAK methods. Height was measured to the nearest millimetre with a calibrated Harpenden stadiometer. Fasted (overnight, 10-15 hours), body weight was measured to the nearest 0.01 kg, on calibrated electronic scales (Sauter platform scale E1200, GmbH, Germany). A flexible, non-stretch, anthropometric tape measure (Lufkin W600PM) was used to measure hip (HC) and waist circumference (WC), to the nearest 1 mm. HC and WC measurements were taken at the end of normal expiration, against participants skin. Participants stood in a relaxed position with their arms folded across their chest and feet comfortably apart. Waist measurements were taken at the narrowest part of the waist and hip measurements at the widest part of the buttocks. Two measurements were taken for height, body weight, WC and HC; if the second measurement was not within 1% of the first, a third measurement was taken. If two measurements were taken, the mean value was recorded and the median if three measurements were taken. Actual BMI (kg/m2) was calculated using measured weight and height. Dual-energy x-ray absorptiometry (DXA) (Hologic QDR Discovery A, Hologic Inc with APEX V. 3.2 software) was used to assess total and regional BF% (including, android, gynoid, trunk and visceral fat %). Researchers who conducted DXA scanning procedures had Australian and NZ Bone Mineral Society clinical densitometry accreditation (Kindleysides et al., 2019).

A digital blood pressure monitor (Omron HEM-907, Omron Healthcare Inc) was used to measure blood pressure and heart rate in the supine position. Following a 10-minute seated resting period, three consecutive measurements were taken at 1-min intervals; the mean of the second and third measurements was used for analysis (Egan et al., 2010).

Metabolic disease risk factors

All blood measurements were taken in a fasted state (overnight, 10-15 hours) between the hours of 7:30am and 9:00am, by a trained phlebotomist. Blood was drawn (maximum total blood volume of 30 mL) to obtain serum and plasma for analysis of metabolic markers and endocrine

regulators. Immediately following blood collection, an aliquot of ethylenediaminetetraacetic acid (EDTA) whole blood was frozen at -80°C for Glycated haemoglobin (HbA1c analysis). The serum vacutainer was left to stand for 30-60 minutes at room temperature (18°C) before centrifugation. For endocrine regulators, an additional plasma sample (Becton Dickinson vacutainer P800 EDTA, aprotinin, and dipeptidyl peptidase IV) was collected. All vacutainers were placed on wet ice immediately after collection until centrifugation. All EDTA vacutainer tubes (including the remainder of the whole blood) were centrifuged at 3500 rpm for 15 minutes at 4°C within one hour of sample collection. Aliquots of plasma and serum were transferred into pre-labelled 1.5 mL microcentrifuge tubes (Eppendorf® safe-lock PCR clean tubes, Hamburg, Germany) and cryovials (Cryo.S Greiner Bio-One, GmbH) and stored immediately at −80°C (Kindleysides et al., 2019).

Plasma levels of leptin were analysed (Plant & Food Research Mt Albert, Sandringham, NZ), using the MILLIPLEX MAP Human Metabolic Hormone Magnetic Bead Panel 96-Well Metabolism Multiplex Assay (Millipore, USA, Cat # HMHEMAG-34K). Leptin samples were assayed in duplicate and plates were read using the Bioplex 100 Analyzer System (Bio-Rad). The leptin range was 0.37–66.0 ng/mL [interassay CV: 4.1%, intraassay CV: 3.5%] and measurements had acceptable percentage of values above the limit of detection (98.4%).

Total cholesterol and non-esterified fatty acids (NEFA) and triglycerides (Trig), HDL, low density lipoprotein (LDL), C-Reactive Protein (CRP) glucose and insulin, were measured using a Cobas e411 automatic electronic analyser (Roche, New Zealand), with kits supplied by Roche Diagnostics (Mannheim, Germany). Homogenous enzymatic colorimetric was used to measure HDL and LDL, CRP was measured by Particle-enhanced Immunoturbidimetric, glucose was measured using Enzymatic UV assay, and insulin measured by Electrochemiluminescence immunoassay (ECLIA) method (Roche Diagnostics, Mannheim, Germany) using the Cobas e411 analyser (Hitachi High Technologies Corporation, Tokyo, Japan). Inter-assay coefficients of variation for total cholesterol, NEFA, Trig, HDL, LDL, CRP, glucose and insulin were 2.0, 3.4, 0.8, 6.1, 0.9, 3.3, 0.7 and 0.5%, respectively. HbA1c levels were measured by turbidimetric inhibition immunoassay (Roche Diagnostic, Mannheim, Germany) on a Hitachi c311 autoanalyser (Hitachi High Technologies Corporation, Tokyo, Japan). The inter-assay %CV for HbA1c was 1.0%. Insulin resistance was calculated using HOMA of insulin resistance (HOMA-IR) (HOMA-IR = fasting plasma insulin (pmol/l) x fasting blood glucose (mmol/L)/ 22.5) (Wallace et al., 2004).

Statistical analyses

IBM SPSS software for Windows version 24.0 (SPSS Inc, Chicago, IL) was used for all statistical analysis. Statistical significance was determined as $p<0.05$. Normality of data was confirmed using histograms and Kolmogorov-Smirnov tests. Fasting insulin and CRP were logarithmically transformed (ln) to ensure a normal distribution.

For descriptive analysis, participants were categorised into two groups based on ethnicity (Pacific or NZE) and within each, two further subgroups based on the predetermined BF% criteria (low <35 or high ≥35 BF%).

Means and standard deviations (SD) were used to summarise all continuous data, except variables that were logarithmically transformed, which were presented as geometric means (GM) and geometric standard deviations (GSD). Frequencies (%) were reported for categorical variables. Differences between ethnic and BF% groups were measured with one-way ANOVA, with Bonferroni post hoc correction. Separate multiple linear regression models were used to assess the association of sedentary behaviour variables with body composition, with adjustment for age, NZDep2013, dietary energy intake and sleep quality (PSQI) as potential confounders. For assessing the independent association of sedentary behaviour variables with metabolic health markers, further adjustment for BF% group was conducted. Regression analyses were conducted separately for NZE and Pacific participants. Regression coefficients (β) obtained from log-transformed data represent relative differences and were expressed as a ratio (by using e^{β}). We present results for both unadjusted (referred to as model 1 in the Tables) and adjusted (for age, deprivation, BF% group, dietary energy intake and sleep quality (PSQI)) analyses (model 2). Differences between ethnic and BF% groups were measured with one-way ANOVA, with Bonferroni post hoc correction. Due to NZE women being significantly older ($p<0.05$) and less deprived ($p<0.05$) than Pacific women and considering age and deprivation are negatively associated with metabolic health (Ministry of Health New Zealand, 2021), all further analysis was carried out controlling for age and deprivation index (NZDep2013). Further, poor sleep behavior and increased dietary energy intake have been associated with SB as well as increased BF% and reduced metabolic health, (Garaulet et al., 2011, Sullivan, 2016, Vereecken et al., 2006), therefore, further analysis also controlled for sleep quality using the PSQI and dietary energy intake using the 5DFR.

5.3 Results

Participant characteristics

Analyses were conducted on 278 women (Figure 5.1). General study population characteristics and metabolic disease markers are presented in Table 5.1. Pacific women were significantly younger ($p<0.05$) and were more deprived (i.e. had higher NZDep2013 scores) than NZE women ($p<0.05$). There was no difference in age when comparing BF% groups among Pacific women, however NZE women in the low-BF% group were younger than their high-BF% counterparts ($p<0.05$). There was no difference in level of deprivation when comparing BF% groups within each ethnic group.

Body composition

Measurements of weight, WC and HC were significantly higher among Pacific women compared to NZE women ($p<0.05$; Table 5.1). There was no difference in total or regional BF% between NZE and Pacific women in the high-BF% groups; however, when comparing the low-BF% groups, NZE women had lower WC, whole body, android and visceral fat percentages ($p<0.05$).

Biomarkers of metabolic health

Pacific women with a high-BF% had significantly higher HbA1c, insulin, HOMA-IR, and lower LDL, total cholesterol and CRP compared to their NZE counterparts. Pacific women with a low-BF% had significantly higher HbA1c, insulin, blood glucose, and lower HOMA-IR, HDL when compared to NZE women with a low-BF%. Pacific women with a high-BF% had significantly higher HbA1c, insulin, HOMA-IR, leptin, trig and lower HDL compared to Pacific women with low-BF%, however no difference in glucose or CRP was observed. NZE women with high-BF% had significantly higher HbA1c, glucose, insulin, HOMA-IR, leptin, LDL, total cholesterol, trig, and lower HDL compared to NZE women with low-BF%.

Table 5.1 Demographic of population, by body fat % group and ethnicity

Characteristic	Pacific		NZE	
	<35% body	≥35% body	<35% body	≥35% body
N	62	57	85	74
BMI (kg/m^2)	25.8 (3.9)†^	35.6 (6.0)	22.5 (2.1)	33.7 (3.8)
Age (years)	25 (7)^	25 (6)^	30 (7)†	33 (7)
Anthropometry				
Weight (kg)	73.5 (11.8)†^	99.8 (18.0)	63.1 (7.6)†	94.4 (12.3)
Height (cm)	169 (6.61)	168 (7.33)	167 (5.61)	167 (7.05)
Waist circumference	79.6 (7.95)†^	98.5 (13.1)	73.2 (5.75)†	98.0 (9.92)
Hip circumference (cm)	105 (7.11)†^	121 (10.7)	98.0 (6.75)†	120 (8.75)
Systolic blood pressure	111 (9.50)†	119 (11.4)	113 (10.6)†	120 (13.7)
Heart rate (beats/minute)	69.1 (11.4)	70.4 (11.1)	67.1 (11.4)	74.3 (12.0)
Whole BF% (%)	30.2 (3.09)†^	39.5 (3.41)	27.8 (4.46)†	41.1 (3.78)
Android fat mass (%)	29.5 (5.16)†^	41.5 (4.74)	24.8 (5.18)†	41.5 (4.90)
Gynoid fat mass (%)	35.0 (3.36)†	40.9 (3.51)	33.3 (4.20)†	42.5 (4.19)
Visceral fat (%)	27.2 (5.59)†^	39.6 (4.94)	22.2 (5.91)†	39.7 (5.15)
Sleep quality				
PSQI global score	6 (3)	6 (3)	5 (2)†	7 (3)
Socioeconomic				
NZDep2013	7 (3)^	8 (2)^	4 (2)	5 (2)
Carbohydrate				
Glucose (mmol/L)	5.31 (0.47)^	5.48 (0.51)	5.12 (0.31)†	5.52 (0.46)
HbA1c (mmol/mol)	32.2 (1.98)†^	34.2 (2.93)^	30.4 (2.19)†	31.6 (2.81)
HOMA-IR	21.4 (19.9)†^	155 (126)^	44.4 (19.1)†	89.9 (48.3)
Lipid profile				
Chol (mmol/L)	4.61 (0.72)	4.72 (0.77)^	4.92 (0.86)†	5.40 (1.18)
HDL (mmol/L)	1.57 (0.32)†^	1.39 (0.31)	1.84 (0.37)†	1.50 (0.32)
LDL (mmol/L)	2.85 (0.67)	2.99 (0.73)^	2.89 (0.83)†	3.53 (1.16)
NEFA (mmol/L)	0.66 (0.32)	0.80 (0.36)	0.67 (0.32)	0.77 (0.38)
Trig (mmol/L)	0.90 (0.35)†	1.24 (0.63)	0.78 (0.28)†	1.20 (0.51)
Endocrine regulators				
Leptin (pg/ml)	9471 (6605)†	23108 (10856)	6037 (4166)†	26852
Insulin (pmol/l)#	72.5 (1.74)†^	123 (1.93)^	40.7 (1.51)†	79.8 (1.61)
Inflammation marker				
CRP (mg/L)#	0.49 (2.53)	1.18 (2.70)^	0.74 (2.81)	2.62 (2.52)

Values are presented as Mean (standard deviation) unless otherwise specified; # = geometric mean (standard deviation of geometric mean); † $p < 0.05$ for test comparing body fat groups within ethnic group. ^$p < 0.05$ for test comparing between ethnic groups within body fat group; BMI, Body mass index. PSQI, Pittsburgh Sleep Quality Index. HbA1c, fasting blood glycated haemoglobin. CRP, fasting C Reactive Protein. Chol, fasting blood total cholesterol. HDL, fasting High-density lipoprotein. LDL, fasting Low-density lipoprotein. NEFA , fasting non-esterfied fatty acids. Trig, fasting triglycerides. NZDep2013, NZDep2013 index of socioeconomic deprivation.

Sedentary behaviour

There was no difference in total sedentary time when comparing Pacific and NZE women with high-BF%. Pacific women with low-BF% spent more time sedentary (10.4 hr/day) compared to NZE women with low-BF% (9.69 hr/day) ($p<0.05$) (Table 5.2).

There was no difference in maximum sedentary bout length when comparing Pacific and NZE women within BF% groups. Pacific women's maximum sedentary bout length did not differ between BF% groups (high-BF% 54.4 min and low-BF% 56.5 min). NZE women with a high-BF% had a significantly higher maximum sedentary bout length when compared to NZE women with a low-BF% (59.9 min versus 50.1 min, $p<0.05$).

NZE women with a high-BF% had a higher ($p<0.05$) weighted median sedentary bout length and higher fragmentation index (20.1 min and 0.41 respectively), compared to their Pacific high-BF% group counterparts (18.3 and 0.31 respectively) and their NZE low-BF% counterparts (17.8 min and 0.31 respectively) (Table 5.2). There was no significant difference in weighted median sedentary bout length or fragmentation index between Pacific women with high and low-BF%.

There was no significant difference between all four groups in time spent in light PA. On average Pacific high-BF% women spent 11.2 mins less in MVPA than their NZE counterparts (19.2 versus 30.4 min, $p<0.05$) and Pacific low-BF% women spent 15.3 minutes less in MVPA than their NZE counterparts (23.8 versus 39.1 min, $p<0.05$) . NZE women with high-BF% spent significantly less time in MVPA (30.4 min) than those with low-BF% (39.1 min), however, there were no difference between Pacific women with high- and low-BF%.

Table 5.2 Sedentary behaviour and physical activity of population, by body fat % group and ethnicity

Characteristic	Pacific		NZE	
	<35% body fat	≥35% body fat	<35% body fat	≥35% body fat
N	62	57	85	74
Accelerometery				
Valid wear time per day (min/day) removed	949 (68.1)	953 (82.9)	930 (56.3)	930 (58.6)
Standardised total sedentary time* (hr/day)	10.4 (1.38)^	9.93 (1.59)	9.69 (1.32)	9.96 (1.58)
Light physical activity (min/day)	310 (79.3)	344 (95.9)	328 (79.5)	320 (86.0)
MVPA (min/day)	23.8 (16.3)^	19.2 (15.1)^	39.1 (18.4)†	30.4 (19.2)
Daily average of sedentary breaks (min)	1178 (130)	1180 (120)	1168 (106)†	1107 (95.5)
Average length of sedentary breaks (min)	129 (51.9)	151 (112)	147 (108)	104 (49.6)
Maximum length of sedentary breaks (min)	989 (414)	1041 (473)	958 (430)	857 (442)
Fragmentation Index	0.31 (0.09)	0.31 (0.10)^	0.31 (0.11)†	0.41 (0.12)
Average length of sedentary bouts (min)	18.2 (1.72)	17.9 (2.10)	17.5 (2.10)†	18.9 (2.44)
Maximum length of sedentary bouts (min)	56.5 (19.8)	54.4 (17.8)	50.1 (12.6)†	59.9 (18.5)
Daily average of sedentary bouts (min)	194 (73.6)	187 (75.2)^	181 (79.3)†	241 (99.0)
Weighted median sedentary bout length (min)	19.2 (3.52)	18.3 (2.85)^	17.8 (3.43)†	20.1 (4.10)

Values are presented as Mean ± standard deviation unless otherwise specified; † $p < 0.05$ for test comparing body fat groups within ethnic group. * Standardised to a 16 hour day. ^$p < 0.05$ for test comparing between ethnic groups within body fat group;. MVPA, moderate to vigorous physical activity.

Total sedentary time was not associated with WC, HC, weight or heart rate in either ethnic group. Among NZE women, every one hour increase in total sedentary time was associated with a 0.8% reduced gynoid % fat ($p<0.05$); this was not the case among Pacific women. This association remained after controlling for age, deprivation index, sleep quality and diet ($p<0.05$) (Table 5.3). Total sedentary time was not associated with other BF% variables in either ethnic group.

Total sedentary time was not associated with fasting plasma insulin, glucose, HOMA-IR, leptin, and all markers of lipid metabolism in either ethnic group (Table 5.3). Among NZE women, every one hour increase in sedentary time was associated with a reduction of 0.35 mmol/mol in HbA1c ($p<0.05$) and an increase in CRP of 1.14 mg/L ($p<0.05$). Results for adjusted and unadjusted analyses were highly comparable.

Every one hour increase in sedentary time was significantly associated with lower systolic and diastolic blood pressure among Pacific women: -1.5 mgH and -1.1 mgH respectively, adjusted analyses (Table 5.3, model 2). There was no association between sedentary time and blood pressure among NZE women.

Table 5.3 Association between total sedentary time per day (h/day) (adjusted to a 16hr day), and markers of cardiometabolic health and body composition

Biomarker	Pacific		NZE	
	Model 1 (n=119) Difference [β (95% CI)] per one unit increase	**Model 2 (n=114)** Difference [β (95% CI)] per one unit increase	**Model 1 (n=159)** Difference [β (95% CI)] per one unit increase	**Model 2 (n=158)** Difference [β (95% CI)] per one unit increase
Endocrine				
HbA1c (mmol/mol)	-0.003 (-0.334, 0.327)	0.126 (-0.188, 0.440)	-0.327 (-0.599, -0.054)*	-0.345 (-0.621, -0.070)*
Glucose (mmol/L)	0.010 (-0.050, 0.071)	0.024 (-0.038, 0.086)	-0.016 (-0.063, 0.031)	-0.033 (-0.076, 0.009)
HOMA-IR	1.719 (-2.057, 5.495)	2.679 (-1.056, 6.414)	0.133 (-1.105, 1.371)	-0.044 (-0.446, 0.358)
Leptin (pg/ml)	-579 (-1986, 827)	307 (-881, 1495)	550 (-870, 1970)	-69 (-1164, 1025)
Chol (mmol/L)	0.001 (-0.090, 0.092)	0.020 (-0.069, 0.109)	-0.029 (-0.142, 0.085)	-0.026 (-0.141, 0.090)
NEFA (mmol/L)	-0.035 (-0.076, 0.007)	0.025 (-0.067, 0.017)	0.014 (-0.024, 0.052)	-0.002 (-0.041, 0.037)
Trig (mmol/L)	0.025 (-0.039, 0.090)	0.044 (-0.018, 0.107)	0.024 (-0.025, 0.073)	0.001 (-0.043, 0.046)
HDL (mmol/L)	-0.019 (-0.059, 0.021)	-0.027 (-0.066, 0.011)	-0.028 (-0.069, 0.014)	-0.006 (-0.045, 0.032)
LDL (mmol/L)	0.012 (-0.074, 0.097)	0.031 (-0.054, 0.116)	-0.014 (-0.127, 0.100)	-0.019 (-0.132, 0.094)
	Ratio (95% CI) per one unit increase	Ratio (95% CI) per one unit increase	Ratio (95% CI) per one unit increase	Ratio (95% CI) per one unit increase
Insulin† (uU/ml)	1.022 (0.942, 1.108)	1.045 (0.968, 1.127)	1.042 (0.980, 1.106)	0.998 (0.950, 1.047)
CRP† (mg/L)	0.986 (0.866, 1.123)	1.050 (0.933, 1.183)	1.197 (1.059, 1.355)**	1.141 (1.022, 1.273)*
Anthropometry	Difference [β (95% CI)] per one unit increase	Difference [β (95% CI)] per one unit increase	Difference [β (95% CI)] per one unit increase	Difference [β (95% CI)] per one unit increase
Systolic blood pressure	-1.937 (-3.243, -	-1.547 (-2.801, -0.292)*	-0.018 (-1.359, 1.359)	-0.122 (-1.514, 1.269)
Diastolic blood pressure	-1.482 (-2.658, -0.305)*	-1.056 (-2.092, -0.021)*	0.868 (-0.231, 1.967)	0.603 (-0.428, 1.634)
Heart rate (bpm)	0.289 (-1.143, 1.721)	0.081 (-1.334, 1.496)	1.235 (-0.152, 2.621)	0.980 (-0.388, 2.349)
Body composition				
Weight (Kg)	-2.308 (-4.724, 0.108)	-2.020 (-4.502, 0.462)	0.831 (-1.220, 2.882)	1.104 (-0.848, 3.056)
Waist circumference (cm)	-1.159 (-2.892, 0.574)	-0.858 (-2.604, 0.887)	-0.399 (-1.995, 1.197)	0.046 (-1.445, 1.537)
Hip circumference (cm)	-1.40 (-2.86, 0.06)	-1.24 (-2.74, 0.25)	0.67 (-0.80, 2.14)	0.88 (-0.51, 2.28)
Trunk fat %	-0.23 (-1.10, 0.64)	-0.12 (-1.02, 0.77)	0.70 (-0.28, 1.69)	0.78 (-0.19, 1.74)

Biomarker	Pacific		NZE	
	Model 1 (n=119)	Model 2 (n=114)	Model 1 (n=159)	Model 2 (n=158)
Android % fat	-0.25 (-1.20, 0.71)	-0.11 (-1.09, 0.88)	0.71 (-0.34, 1.77)	0.70 (-0.34, 1.74)
Gynoid % fat	-0.00 (-0.56, 0.55)	-0.02 (-0.59, 0.56)	0.76 (0.10, 1.42)*	0.77 (0.11, 1.43)*
Visceral fat %	-0.21 (-1.21, 0.79)	-0.08 (-1.11, 0.95)	0.83 (-0.29, 1.95)	0.78 (-0.32, 1.88)
Total body fat %	-0.23 (-0.93, 0.46)	-0.16 (-0.88, 0.56)	0.75 (-0.09, 1.60)	0.80 (-0.03, 1.63)

Model 1: Unadjusted

Model 2: Adjusted for age, deprivation index, sleep quality (PSQI global score), dietary energy intake KJ, body fat % group (excluding body composition variables)

*P value <0.05, **P value ≤0.01, ***P value ≤0.001. † data has been log transformed (*ln*). Pacific, participants who identify as Pacific. NZ European, participants who identify as New Zealand European. HDL, fasting High-density lipoprotein. LDL, fasting Low-density lipoprotein. NEFA, fasting non-esterfied fatty acids. Trig, fasting triglycerides.

Regression coefficients (β) obtained from log-transformed data represent relative differences and are expressed as a ratio (by using e^{β}).

Among NZE but not Pacific women, several significant associations were observed between maximum sedentary bout length and body composition (total and regional BF%, weight, WC and HC) (Table 5.4). After adjusting for confounders, significant associations between weight, WC and HC remained but reduced in size. Among NZE women, every one minute increase in maximum sedentary bout length was associated with a 0.3 kg higher body weight ($p<0.05$), 0.1 cm higher WC ($p\leq0.001$), and 0.2 cm higher HC ($p<0.05$). Associations with all BF% measures were no longer observed after adjusting for confounders.

No significant associations were found between maximum sedentary bout length and biomarkers of metabolic health among Pacific women (Table 5.4). This was also the case among NZE women, except for LDL. Every one minute increase in maximum sedentary bout length was associated with a 0.01 mmol/L higher LDL level ($p<0.05$). However, after adjusting for confounders, this association was no longer observed.

Table 5.4 Association between maximum sedentary bout length and markers of cardiometabolic health and body composition

Biomarker	Pacific		NZE	
	Model 1 (n=119)	**Model 2 (n=114)**	**Model 1 (n=159)**	**Model 2 (n=158)**
	Difference [β (95% CI)] per one unit increase	Difference [β (95% CI)] per one unit increase	Difference [β (95% CI)] per one unit increase	Difference [β (95% CI)] per one unit increase
Endocrine				
HbA1c (mmol/mol)	-0.001 (-0.027, 0.025)	-0.001 (-0.026, 0.023)	0.007 (-0.017, 0.032)	-0.008 (-0.033, 0.017)
Glucose (mmol/L)	0.001 (-0.003, 0.006)	0.001 (-0.004, 0.006)	0.003 (-0.001, 0.007)	-0.002 (-0.006, 0.002)
HOMA-IR	0.195 (-0.104, 0.494)	0.199 (-0.095, 0.493)	0.016 (-0.094, 0.126)	-0.093 (-0.188, 0.002)
Leptin (pg/ml)	-13 (-122, 97)	11 (-80, 101)	133 (-4, 270)	-38 (-147, 71)
TNF α (pg/ml)	0.034 (-0.426, 0.494)	0.025 (-0.009, 0.058)	0.304 (-0.233, 0.841)	-0.007 (-0.028, 0.015)
Chol (mmol/L)	0.002 (-0.006, 0.009)	0.002 (-0.005, 0.009)	0.009 (-0.001, 0.019)	0.004 (-0.006, 0.015)
NEFA (mmol/L)	-0.002 (-0.005, 0.002)	-0.001 (-0.005, 0.002)	0.001 (-0.002, 0.004)	0.000 (-0.003, 0.004)
Trig (mmol/L)	0.001 (-0.004, 0.006)	0.001 (-0.004, 0.006)	0.002 (-0.003, 0.006)	-0.002 (-0.006, 0.002)
HDL (mmol/L)	0.000 (-0.003, 0.003)	5.003E-5 (-0.003,	-0.003 (-0.007, 0.001)	0.000 (-0.004, 0.003)
LDL (mmol/L)	0.003 (-0.004, 0.009)	0.003 (-0.004, 0.010)	0.011 (0.001, 0.021)*	0.005 (-0.005, 0.016)
	Ratio (95% CI) per one unit increase	Ratio (95% CI) per one unit increase	Ratio (95% CI) per one unit increase	Ratio (95% CI) per one unit increase
Insulin† (uU/ml)	1.002 (0.996, 1.009)	1.003 (0.997, 1.009)	1.003 (0.998, 1.008)	0.997 (0.993, 1.001)
CRP† (mg/L)	0.996 (0.986, 1.006)	0.996 (0.987, 1.005)	1.009 (0.998, 1.020)	0.999 (0.989, 1.009)
Anthropometry	Difference [β (95% CI)] per one unit increase	Difference [β (95% CI)] per one unit increase	Difference [β (95% CI)] per one unit increase	Difference [β (95% CI)] per one unit increase
Systolic blood pressure (mgH)	0.002 (-0.106, 0.109)	0.017 (-0.084, 0.118)	0.031 (-0.091, 0.154)	-0.032 (-0.158, 0.094)
Diastolic blood pressure (mgH)	-0.013 (-0.109, 0.083)	0.004 (-0.079, 0.086)	0.096 (-0.001, 0.194)	0.014 (-0.080, 0.108)
Pulse (bpm)	-0.039 (-0.168, 0.090)	-0.013 (-0.138, 0.112)	0.028 (-0.096, 0.151)	-0.034 (-0.160, 0.092)
Body composition				
Weight (Kg)	-0.097 (-0.291, 0.097)	-0.095 (-0.293, 0.103)	0.303 (0.126, 0.479)**	0.247 (0.078, 0.416)**

Biomarker	Pacific		NZE	
	Model 1 (n=119)	**Model 2 (n=114)**	**Model 1 (n=159)**	**Model 2 (n=158)**
Waist circumference (cm)	-0.058 (-0.197, 0.080)	-0.055 (-0.193, 0.084)	0.200 (0.061, 0.338)**	0.140 (0.010, 0.270)*
Hip circumference (cm)	-0.055 (-0.173, 0.062)	-0.047 (-0.166, 0.072)	0.208 (0.080, 0.335)**	0.161 (0.039, 0.282)*
Trunk fat %	-0.03 (-0.10, 0.04)	-0.02 (-0.09, 0.05)	0.23 (0.03, 0.20)**	0.07 (-0.01, 0.16)
Android % fat	-0.02 (-0.09, 0.06)	-0.01 (-0.09, 0.06)	0.13 (0.04, 0.22)**	0.09 (-0.01, 0.18)
Gynoid % fat	-0.02 (-0.06, 0.03)	-0.01 (-0.06, 0.03)	0.06 (0.00, 0.12)*	0.04 (-0.02, 0.10)
Visceral fat %	-0.02 (-0.10, 0.06)	-0.01 (-0.09, 0.07)	0.13 (0.03, 0.23)*	0.09 (-0.01, 0.19)
Total body fat %	-0.02 (-0.07, 0.04)	-0.02 (-0.07, 0.04)	0.10 (0.02, 0.17)*	0.07 (-0.01, 0.14)

Model 1: Unadjusted

Model 2: Adjusted for age, deprivation index, sleep quality (PSQI global score), dietary energy intake KJ, body fat % group (excluding body composition variables)

*P value <0.05, **P value ≤0.01, **P value ≤0.001. † data has been log transformed (*ln*). Pacific, participants who identify as Pacific. NZ European, participants who identify as New Zealand European. HDL, fasting High-density lipoprotein. LDL, fasting Low-density lipoprotein. NEFA, fasting non-esterfied fatty acids. Trig, fasting triglycerides.

Regression coefficients (β) obtained from log-transformed data represent relative differences and were expressed as a ratio (by using e^{β}).

Weighted median sedentary bout length was not associated with any body composition or endocrine measure among Pacific women (Table 5.5). Among NZE women, every one minute increase in weighted median sedentary bout length was associated with 1.1 kg significantly higher body weight, 0.62 cm WC, 0.79 cm HC, and higher BF measures; 0.39% higher gynoid %fat and 0.39% total body, 0.42% trunk, 0.41% android and 0.42% visceral % fat (Table 5.5, model 2).

Among NZE but not Pacific women, unadjusted analysis showed every one minute increase in weighted median bout length was associated with significant increases of 2.4% in insulin , 0.02 mmol/L glucose, 0.05 mmol/L LDL, 657 pg/mL leptin, and 0.4 mgH diastolic blood pressure (Table 5.5, model 1), after controlling for confounders, significant associations were no longer observed (Table 5.5).

Table 5.5 Association between weighted median sedentary bout length (min), and markers of cardiometabolic health and body composition

Biomarker	Pacific		NZE	
	Model 1 (n=119)	**Model 2 (n=114)**	**Model 1 (n=159)**	**Model 2 (n=158)**
Endocrine	Difference [β (95% CI)] per one unit	Difference [β (95% CI)] per one unit	Difference [β (95% CI)] per one unit increase	Difference [β (95% CI)] per one unit increase
HbA1c (mmol/mol)	-0.006 (-0.158, 0.145)	0.017 (-0.126, 0.160)	0.042 (-0.061, 0.145)	-0.027 (-0.134, 0.079)
Glucose (mmol/L)	0.013 (-0.015, 0.041)	0.015 (-0.013, 0.044)	0.021 (0.003, 0.038)*	0.001 (-0.015, 0.017)
HOMA-IR	0.516 (-1.231, 2.263)	0.991 (-0.736, 2.718)	0.362 (-0.094, 0.818)	-0.044 (-0.446, 0.358)
Leptin (pg/ml)	-456 (-1087, 175)	-201 (-728, 327)	657 (133, 1181)*	15 (-404, 434)
TNF α (pg/ml)	0.0548 (-0.186, 0.186)	0.008 (-0.189, 0.204)	-0.008 (-0.084, 0.069)	-0.018 (-0.102, 0.065)
Chol (mmol/L)	0.021 (-0.021, 0.063)	0.027 (-0.014, 0.067)	0.037 (-0.005, 0.078)	0.016 (-0.027, 0.060)
NEFA (mmol/L)	-0.002 (-0.022, 0.017)	0.000 (-0.020, 0.020)	0.001 (-0.013, 0.016)	-0.001 (-0.016, 0.014)
Trig (mmol/L)	-0.002 (-0.032, 0.028)	0.006 (-0.024, 0.035)	0.005 (-0.013, 0.024)	-0.010 (-0.027, 0.007)
HDL (mmol/L)	0.004 (-0.014, 0.023)	-0.012 (-0.018, 0.018)	-0.011 (-0.026, 0.004)	0.000 (-0.015, 0.014)
LDL (mmol/L)	0.022 (-0.017, 0.061)	0.028 (-0.011, 0.067)	0.045 (0.003, 0.086)*	0.020 (-0.023, 0.062)
	Ratio (95% CI) per one unit increase	Ratio (95% CI) per one unit increase	Ratio (95% CI) per one unit increase	Ratio (95% CI) per one unit increase
Insulin† (pmol/l)	1.001 (0.964, 1.039)	1.015 (0.980, 1.051)	1.024 (1.001, 1.047)*	1.002 (0.098, 1.020)
CRP† (mg/L)	0.968 (0.911, 1.026)	0.989 (0.937, 1.045)	1.039 (0.991, 1.089)	0.998 (0.957, 1.042)
Anthropometry	Difference [β (95% CI)] per one unit increase	Difference [β (95% CI)] per one unit increase	Difference [β (95% CI)] per one unit increase	Difference [β (95% CI)] per one unit increase
Systolic blood pressure	-0.066 (-0.691, 0.559)	0.182 (-0.409, 0.774)	0.089 (-0.422, 0.601)	-0.137 (-0.667, 0.392)
Diastolic blood pressure	-0.270 (-0.825, 0.286)	-0.006 (-0.492, 0.480)	0.418 (0.012, 0.824)*	0.085 (-0.309, 0.479)
Heart rate (bpm)	0.141 (-0.567, 0.850)	0.295 (-0.400, 0.990)	0.395 (-0.117, 0.906)	0.230 (-0.289, 0.748)
Body composition				
Weight (Kg)	-0.798 (-1.921, 0.325)	-0.905 (-2.044, 0.234)	1.333 (0.599, 2.066)***	1.089 (0.380, 1.799)**
Waist circumference (cm)	-0.512 (-1.313, 0.289)	-0.529 (-1.327, 0.269)	0.892 (0.316, 1.468)**	0.617 (0.071, 1.164)*
Hip circumference (cm)	-0.559 (-1.238, 0.119)	-0.605 (-1.289, 0.079)	0.980 (0.454, 1.506)***	0.785 (0.278, 1.291)**
Trunk fat %	-0.18 (-0.58, 0.21)	-0.19 (-0.59, 0.22)	0.58 (0.22, 0.93)**	0.42 (0.06, 0.78)*
Android % fat	-0.16 (-0.60, 0.28)	-0.17 (-0.62, 0.28)	0.58 (0.20, 0.97)**	0.41 (0.02, 0.79)*
Gynoid % fat	-0.15 (-0.41, 0.10)	-0.16 (-0.42, 0.11)	0.40 (0.16, 0.64)**	0.30 (0.05, 0.54)*
Visceral fat %	-0.17 (-0.62, 0.29)	-0.17 (-0.64, 0.29)	0.60 (0.20, 1.01)**	0.42 (0.01, 0.82)*
Total body fat %	-0.18 (-0.50, 0.14)	-0.18 (-0.51, 0.14)	0.53 (0.22, 0.83)**	0.39 (0.08, 0.70)*

Model 1: Unadjusted
Model 2: Adjusted for age, deprivation index, sleep quality (PSQI global score), dietary energy intake KJ, body fat % group (excluding body composition variables)
*P value <0.05, *P value ≤0.01, **P value ≤0.001. † data has been log transformed (*ln*). Pacific, participants who identify as Pacific. NZ European, participants who identify as New Zealand European. HDL, fasting High-density lipoprotein. LDL, fasting Low-density lipoprotein. NEFA, fasting non-esterfied fatty acids. Trig, fasting triglycerides.
Regression coefficients (β) obtained from log-transformed data represent relative differences and were expressed as a ratio (by using e^{β}).

5.4 Discussion

Sedentary behaviour was common amongst our participants, with a daily average of 9.93 and 10.4 hours for Pacific women with a high- and low-BF% respectively, and 9.96 and 9.69 hours for NZE women with a high- and low-BF% respectively; comparable to Canadian adults (9-10 hours per day of SB (Prince et al., 2020). However, accelerometery data from the U.S. National Health and Nutrition Examination Survey (NHANES) indicate that approximately 55% of their population's awake time (7.7 hours/day) is spent being sedentary (Matthews et al., 2008), which is considerably less than our population. Using accelerometery data, Beale et al. (2020) investigated a population of NZ women (including Pacific and NZE), and found that participants spent an average of 7 hours 42 min/day in SB, over two hours less than women in this study. However Beale et al. (2020) did not report accelerometer wear time and also included Māori women, which may have contributed to the difference in sedentary time observed.

We found in NZE, but not Pacific women, the pattern in which sedentary time was accumulated, expressed as weighted median sedentary bout length, was associated with a higher total, gynoid, android, trunk and visceral BF% as well as body weight, waist- and hip circumferences. Additionally, maximum sedentary bout length was associated with a higher body weight, waist- and hip circumferences and total sedentary time was associated with a higher gynoid BF%. These findings support results from previous studies reporting less prolonged or more interrupted sedentary accumulation patterns (i.e., more regular breaks, shorter sedentary bouts), are associated with lower BMI or BF% (Werneck et al., 2019, Healy et al., 2008, Chastin et al., 2015, Huang et al., 2021). We observed that after adjustment for potential confounders, associations between both weighted median sedentary bout length and total sedentary time and body composition (BF%, weight, waist and hip circumference) reduced in magnitude. Similar results were also observed by Heinonen et. al. (2013), who found that TV viewing was strongly associated with larger WC and BMI and Werneck et al. (2019) who found total SB and bouts of sedentary time between five and 14 minutes were associated with a greater adiposity (BMI, WC and sum of skinfolds). However, after controlling for a range of potential genetic and lifestyle confounders, including diet (Heinonen et al., 2013) and age (Heinonen et al., 2013, Werneck et al., 2019) these associations were less pronounced or disappeared. This indicates that if detrimental effects of SB on body composition exist, it may be partly mediated by other factors, such as age, diet, level of deprivation and sleep.

In the current study there was no association between Pacific women's CRP and SB, whereas a significant association was observed between total sedentary time and increased CRP ($p<0.05$) among NZE women. Following adjustment for confounders (including age, deprivation, sex and BMI) others have reported total sedentary time to be detrimentally associated with CRP in both general populations (Healy et al., 2011) and population at high risk of T2D (Henson et al., 2013). Among adults with newly diagnosed T2D, Falconer et al. (2014) found reduced sedentary time over a six month period was associated with lower levels of CRP in women. Given CRP is an inflammatory marker associated with increased risk of poor metabolic health (Kaptoge et al., 2010) and despite having a similar total BF%, and spending similar (high-BF group) or more (low-BF% group) time in SB, our finding that Pacific women had significantly lower CRP levels compared to NZE women is important. Inflammation may be an additional pathway through which increased sedentary time may impact on metabolic risk in women, especially NZE, when compared to Pacific women.

Total sedentary time was associated with significantly lower systolic and diastolic blood pressure among Pacific but not NZE women (-1.5 mgH and -1.1 mgH respectively ($p<0.05$) (Table 5.3, model 2), however this association is small in magnitude and is unlikely to be deemed clinically significant. Previous research also using objectively measured, accelerometer-derived sedentary data, found non-active sitting to be positively correlated ($p\leq 0.001$) with various markers of metabolic health (higher BMI, BF%, body mass, fat mass, leptin, and lower HDL-cholesterol ($p=0.031$)) whereas active sitting was negatively correlated ($p\leq 0.001$) with various markers of metabolic health (lower BMI, BF%, body mass, fat mass and leptin, and higher HDL-cholesterol ($p=0.035$) (Beale et al., 2020). Hagger-Johnson et al. (2016) also reported a lower risk of mortality in women who fidget while sitting compared to those who do not. Therefore, if the association between increased sedentary time and decreased blood pressure is in fact a true relationship, it may be that Pacific women in this study are engaging in SB's with MET values towards the upper end of the sedentary range, such as active sitting (e.g. typing on a computer), or any other physical activities not detected by a hip-mounted accelerometer (e.g., stationary seated exercise and isometric physical activities) (Pedišić and Bauman, 2015, Herman Hansen et al., 2014, Kozey et al., 2010, Ellis et al., 2016), and impacting metabolic health. This suggests future research on SB may need to include domains (e.g. leisure, occupational, etc.), types (e.g. sitting on couch watching TV or typing on a computer) and context of SB to ensure an accurate interpretation of the results.

Strengths and limitations of this study

This study has several strengths. The study included an extensive data collection protocol; a range of outcome measures including body composition and metabolic health biomarkers, and important potential confounders including age, level of deprivation, dietary energy intake, sleep quality and BF% were collected. Sedentary time was measured objectively using accelerometers. In comparison to self-report measures, it is likely that this resulted in recording more valid and comparable levels of sedentary time between ethnic groups. However, we acknowledge when wearing an accelerometer some individuals may change their PA behaviours, particularly on the first day of monitoring. Equally, some remove the device early on the last day of wearing; both of these issues threatening the validity of the data (Baumann et al., 2018). To remove this bias, we excluded data from the first and last days of accelerometer monitoring. This also meant all participants had the same start time, regardless of the time of day they received the accelerometer. Reviewing the PA diaries, approximately 20 participants took the accelerometer off for water sports on 1-2 monitoring days for approximately one hour per session, consequently this was marked as non-wear time. Although this is a small portion of total participant wear time, the inability of the accelerometer to detect activity while the wearer participating in water sports may have introduced a bias to the estimation of PA. Self-reported PA and SB measures suffer from recall and social desirability bias and it is also possible that linguistic and cultural differences in the interpretation of questions can occur with self-report measures (Hunt and Bhopal, 2004, Sallis and Saelens, 2000). This latter point may be particularly important in research such as ours that is focussed on differences between ethnicities, as interpretation can differ. Nevertheless, objective measures such as the w-GT3X accelerometer lack contextual and domain specific information, concealing ethnic differences in sedentary time domains.

A further strength was the use of DXA for estimating BF%, as it is well recognised for its precision and accuracy (Prior et al., 1997). The accelerometer data collection across a full 24-hour period and over an entire week, in a free-living setting, was important given the variability in activities performed within a day and across a week. This also allowed for the objective measurement of sleep onset and wake times, not compromising the objective nature of the data, and may also have improved wear-time compliance.

The major limitation of the study is the cross-sectional design, meaning causality cannot be inferred. It is possible women with obesity in this study had increased sedentary time because of their increased BF% (i.e. reversed causation). The adjustment for BF% group was necessary

due to the nature of the PROMISE study design. However BF% could be part of the pathway between SB and poor metabolic health. It is therefore possible, the analyses could have been subject to over adjustment. Most research investigating associations between SB and metabolic health also adjust for body composition due to its well established relationship with metabolic health (Helmerhorst et al., 2009, Huang et al., 2021, Green et al., 2014). Green et al. (2014) found SB to be associated with biomarkers of metabolic health such as triglycerides and lipid accumulation product (WC (cm) minus 58 multiplied by triglyceride concentration (mmol L−1)), however these associations were attenuated by body mass or body composition indicating body composition may be an important mediator. Further, as previously mentioned, with the exception of CRP among our NZE women, even in our unadjusted model any significant associations between SB and biomarkers of metabolic health were very small. The positive associations found between NZE women's weighted median sedentary bout length and insulin, glucose, leptin and LDL as well as maximum sedentary bout length and LDL, are smaller than the inter-assay precision of the techniques we used to measure these biomarkers. For example we found significantly higher glucose of 0.02 mmol/L with every additional minute of weighted median sedentary bout length, however our inter-assay CV for glucose is >0.03 mmol/L. Therefore, we cannot conclude that these associations are meaningful and not due to "noise" in the system. As with body composition, after controlling for confounders these associations either dissipated or reduced in magnitude. It is therefore possible that any relationships between SB and these biomarkers of metabolic health are due to residual confounding. The finding of a significant association between total sedentary time and decreased HbAlc among NZE women was also just above the inter-assay precision of the technique used to measure this biomarker, thus may be due to inherent measurement variation. The observed association was also very small in magnitude; for NZE women's HbA1c to decrease by 1 mmol/mol they would need to spend an extra 2.8 hours/day in SB. We acknowledge that small changes to metabolic biomarkers accumulated over time can result in large changes to metabolic health. However, in this instance, it is hard to argue that such a difference is of clinical relevance, considering a change of 10 mmol/mol is required to shift someone from one risk band to another (e.g. from "normal" to "pre-diabetes", or from "pre-diabetes" to "diabetes"; based on the definitions of <40 mmol/mol = normal, 41−49 mmol/mol = pre-diabetes, ≥50 mmol/mol = diabetes (New Zealand Society for the Study of Diabetes, 2011)). Maher et al. (2014) drew attention to the fact that in many studies, investigating the relationship between total sedentary time and markers of metabolic health, associations found are generally weak and small in magnitude, and may not be of major clinical significance.

Finally, our findings are only generalisable to NZE and Pacific, pre-menopausal women, free of chronic disease, therefore future research should be conducted on males and other age groups and ethnic groups, particularly those with high metabolic disease risk.

5.5 Conclusion

Women in this study had high levels of SB that may be a contributor to BF% and/or reduced metabolic health, differing between Pacific and NZE women. Our findings indicate breaking-up prolonged SB may assist in achieving healthier BF%. Our results also suggest inflammation may be an additional pathway through which increased sedentary time may impact on metabolic disease risk in women, especially NZE, when compared to Pacific women. All other associations between SB and other markers of metabolic health were small in magnitude and may not be of major clinical or public health relevance. Prospective longitudinal and randomised controlled studies, investigating the impact of SB on BF% and metabolic health are needed to confirm these results and establish causality.

5.6 References

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Chapter 6: Exploring associations between physical activity and nutrient intake among Pacific and New Zealand European women

Abstract

Aim: To explore associations between meeting the PA guidelines, nutrient intakes and body composition in Pacific and New Zealand European (NZE) women, aged 18-45 years, with low- (<35%) or high-body fat % (BF%) (≥35%).

Methods: Pacific (n=119) and NZE (n=159) women, aged 18-45 years were recruited according to self-reported normal or obese body mass index (BMI). Physical activity (PA) was assessed using eight-day accelerometery. Dietary intake data were collected using a prospective, estimated five-day food record (5DFR). Fasting blood was assessed for biomarkers of metabolic health. Body composition was assessed using BMI, waist circumference, and BF% (whole body dual-energy x-ray absorptiometry). BF% was used to stratify women as low (<35%) or high (≥35%) BF%. Logistic regression analysis was used to assess associations between nutrient intake and PA, whilst controlling for potential confounders.

Results: New Zealand European women (n=159) were significantly older ($p<0.05$) and less deprived ($p<0.05$) compared to Pacific women (n=119). Only 39% of high-BF% and 47% low-BF% Pacific women were meeting the PA guidelines, compared to 65% of the high-BF% and 81% low-BF% NZE women. There were no significant difference in the percentage of participants meeting the PA guidelines when comparing BF% groups within each ethnicity. None of the women consumed more than the NZ carbohydrate guidelines (45-65% of total energy (TE)). Although Pacific women's mean daily starch intake was significantly higher than NZE women, the mean daily starch intake (g/day, and % TE) did not differ between BF% groups. Only the NZE low-BF% group's mean fibre intake was above the recommended daily intake (RDI) of ≥25 g/day, the lowest being 19.2 g/day in the Pacific high-BF% group. Meeting the PA guidelines was not associated with any of the investigated micronutrient dietary intakes (iron, vitamin B_{12} and iodine). The odds of meeting the PA guidelines was lower among women who were in the lowest quartile of fibre intake compared to the top three quartiles (NZE women OR 0.66, p=0.021 and all women OR 0.72, p=0.008). There was a decreased odds of meeting the PA guidelines among women who were in the lowest quartile of polyunsaturated fat intake

when measured as a % of total energy intake (all women OR 0.76, p=0.027 and Pacific women OR 0.67, p=0.030)

Conclusion: There was a lack of association between nutrient intake and meeting the PA guidelines. Focusing on a different approach such as a food-based approach e.g. dietary patterns, may be beneficial to further explore and develop a deeper understanding of the relationship between food choice, PA and metabolic health.

6.1 Introduction

Physical activity (PA), a balanced diet and healthy sleep habits are essential for the maintenance of health and prevention of adverse health outcomes such as obesity (Romieu et al., 2017, Garaulet et al., 2011). Unhealthy diets and physical inactivity are currently two of the major risk factors for non-communicable diseases and global mortality (World Health Organisation, 2017, Afshin et al., 2019). Current global and New Zealand (NZ) dietary guidelines encourage consumption of a wide variety of foods from fruits and vegetables, wholegrains, lean meats and dairy products, whilst limiting saturated fat, added salt and sugars, as well as alcohol (World Helath Organisation, 2015, Willett et al., 2019). Similarly, long-standing PA guidelines include promoting the accumulation of ≥ 30 minutes of moderate or greater intensity activity on ≥ 5 days per week OR 150 minutes of moderate to vigorous PA (MVPA) per week (World Health Organisation, 2020, Ministry of Health New Zealand, 2020, U.S. Department of Health and Human Services, 2018). Alarmingly, a 2018 review including data from across 168 countries and including 1.9 million participants, found the global age standardised prevalence of insufficient PA to be 27.5% (Guthold et al., 2018). Furthermore, the prevalence of physical inactivity was higher among women than men (31.7% and 23.4% respectively) (Guthold et al., 2018).

Alongside other Pacific nations, NZ, has among the highest obesity prevalence in the world (OECD, 2017) with almost one third (31%) of adults (≥15 years) classified according to BMI as obese (Ministry of Health New Zealand, 2021). Obesity is a well-recognised risk factor for the development of various chronic diseases, including cardiovascular disease (CVD), type two diabetes (T2D) and some cancers (Guh et al., 2009). Within high-income countries, those most deprived are more likely to be obese (Pampel et al., 2012). This is evident in NZ, with adults living in the most socioeconomically deprived areas being 1.8 times more likely to be obese than adults living in the least deprived areas (Ministry of Health New Zealand, 2021). Furthermore, NZ adults living in the most deprived areas are significantly less likely (OR:

0.82; $p<0.05$) to meet the NZ PA guidelines (Ministry of Health New Zealand, 2021) compared to those living in the least deprived areas, whilst simultaneously suffering from low food security and consequently poorer dietary intake; for example they are 35% less likely to consume the recommended daily servings of fruit and vegetables (New Zealand Ministry of Health, 2020). NZ Māori (the indigenous population of NZ) and Pacific peoples (people who identify with a Pacific ethnicity (eg Samoan, Tongan, Fijian) with or without other ethnicities, residing in NZ) (Stats New Zealand, 2015) disproportionately occupy low socioeconomic strata in NZ. Concurrently, the obesity prevalence is highest in these ethnic groups, particularly among women: NZ Māori (49%) and NZ Pacific (62%) women compared to NZ European (NZE) /other women (30%) (Ministry of Health New Zealand, 2021).

Physical activity induces changes in hormones implicated in appetite regulation and energy balance (including leptin, ghrelin, peptide YY, glucagon-like peptide-1 and insulin) (Schubert et al., 2014, Gondim et al., 2015). Being habitually physically active allows individuals to have a heightened sensitivity to their appetite regulation, better matching energy intake with energy expenditure (Hopkins and Blundell, 2016) (Dorling et al., 2018).

Whilst research has been carried out investigating the effects of PA on energy and nutrient intakes, the evidence of a direct relationship is mixed (Elder and Roberts, 2007). A better understanding of how meeting the PA guidelines is associated with nutrient intake in different ethnic groups will aid in designing more effective, efficient obesity prevention strategies and public health policies, targeted at population groups most at risk. Therefore the aim of this study was to explore associations between meeting the PA guidelines and nutrient intakes in two groups of healthy Pacific and NZE women aged 18-45 years, and whether this differs between these ethnic groups.

6.2 Materials and Methods

Participants were part of the cross-sectional PRedictors linking Obesity and the gut MIcrobiomE (PROMISE) study. Protocol details have been published for the PROMISE study as a whole elsewhere (Kindleysides et al., 2019), therefore only information relevant to the current chapter is provided here. Briefly, inclusion criteria included: post-menarche and pre-menopausal (as defined by regular menstrual cycles over the last year), Pacific and NZE women (self-identified), aged 18-45 years, who self-reported being in good health. Women who had a BMI (calculated from self-reported height and weight) within the predefined normal or obese BMI ranges (BMI $\geq$18.5 to $<$25.0 kg/m^2 and $\geq$30.0 kg/m^2, respectively) were invited to participate. Recognising people with the same BMI can have heterogeneity of body fat and

metabolic disease risk factors (Oliveros et al., 2014, Kramer et al., 2013, Dickey et al., 1998), participants were subsequently classified as either low-BF% (<35%) or high-BF% (≥35%). The Obesity Medicine Association guidelines (obesity in women >35%) were used to derive these BF% cut-offs (Fitch and Bays, 2022). Exclusion criteria were presence of any diagnosed chronic illness (e.g., T2D and CVD), being pregnant or lactating.

All participants provided written informed consent and attended two clinic appointments, 11-14 days apart. The flow of data collection procedures is outlined in figure 6.1. The Health and Disability Ethics Committees Ethics committee approved the study (HDEC, reference: 16/STH/32) and the trial was prospectively registered at anzctr.org.au (ACTRN12618000432213). Researchers conducted standardised face-to-face interviews (e.g., address, personal/household income, and medication use) to obtain demographic and health information.

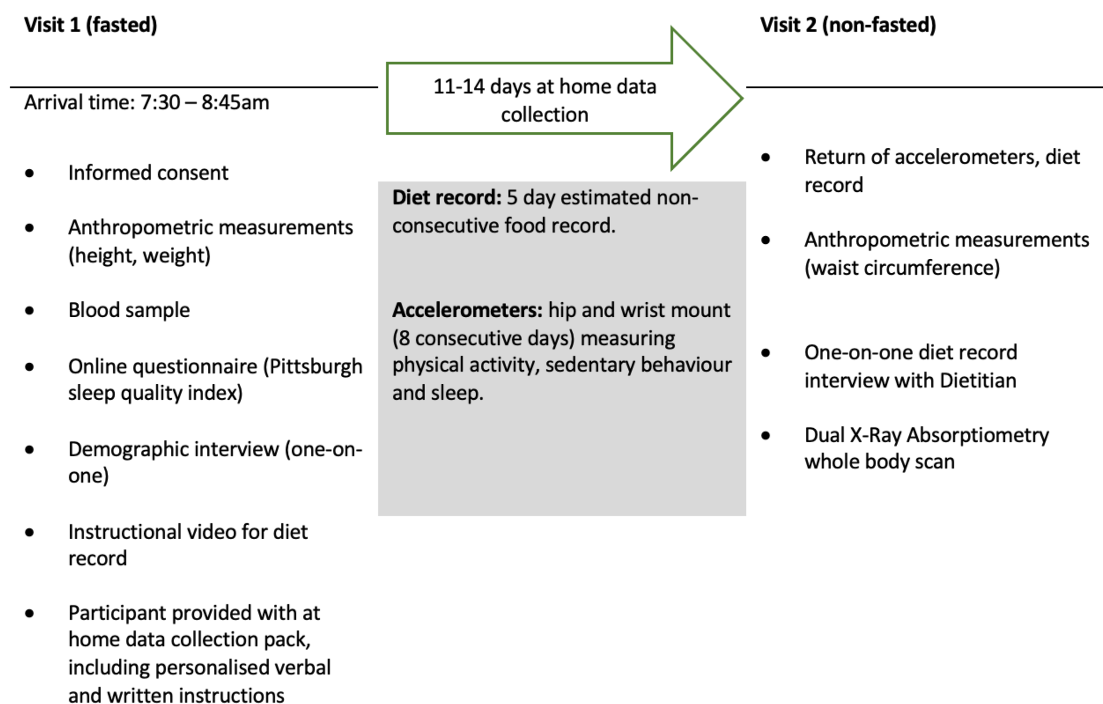


Figure 6.1 Flow of data collection procedures

Physical activity was measured with a w-GT3X tri-axial accelerometer (Actigraph LLC, 2016), worn on the non-dominant hip (Actigraph LLC, 2016) and an Acti-Watch (Micro Motionlogger®) worn on the non-dominant wrist (Ward et al., 2005) (Ancoli-Israel et al., 2003). Devices were set to record epoch length of one minute. Participants were fitted with their accelerometer at clinic visit one. For the following eight days participants wore both devices continuously, except while participating in water activities such as swimming or showering. During the accelerometer wearing period, participants completed a daily PA and sleep diary recording: 1) times the participant removed and put back on accelerometers; what they were doing in the time they were not wearing the accelerometers (e.g swimming, showering) and 2) sleep onset; and wake times for all sleep ≥10 minutes. Participants also marked sleep onset and wake time in the Acti-Watch memory; when they went to bed and turned off their light to sleep, as well as when they woke up, by pressing the Acti-Watch "event" button.

Acti-Life® software (version 6.13.3, Actigraph) was used to process the w-GT3X accelerometer data. The first and last days (days of partial wear) of monitoring were not included in data analysis, due to incomplete data. Data sets were focused on PA using a midnight-midnight 24-h data format. We identified accelerometer epochs occurring during sleep periods using the sleep diaries. Sleep times were confirmed using the ActiWatch data. Sleep times were subsequently recorded, and removed, leaving only epochs that occurred during waking hours.

Non-wear time for the wGT3X was defined as ≥ 60 consecutive minutes of zero epoch counts, allowing for two minutes of counts between zero and 100 vector magnitude units. Data from participants who wore the accelerometer for ≥12 hours/day, on ≥ four days, including at least one weekend day was considered valid (Tudor-Locke et al., 2012). Data that did not meet the aforementioned criteria was considered invalid and removed for analysis. Valid data was used to calculate, mean time (min/day) spent in MVPA (≥2020 counts per minute (cpm)) (Troiano et al., 2008). Vector magnitude mean activity count (cpm) was calculated and used as an indicator of total PA volume (i.e. total daily movement).

To assess sleep quality participants completed the previously validated Pittsburgh Sleep Quality Index (PSQI) questionnaire (Buysse et al., 1989).

Dietary intake data were collected using a prospective, five-day food record (5DFR) on pre-allocated, non-consecutive days, including at least one weekend day (Gibson, 2005). The

5DRF was completed between clinic visits one and two. Estimated rather than weighed food records were used to reduce participant burden (Gibson, 2005, Livingstone and Black, 2003). At clinic visit one, participants received verbal training for filling out the food record and estimating food and beverage portions. Participants were provided with a standardised food portion booklet, accompanied by images of standard household food measures, to help estimate portion sizes. They also watched a 15-minute food record instruction and demonstration video (developed by NZ Registered Dietitians at Massey University), providing them with in-depth detail on how to correctly complete their food record.

Upon return at visit two, each participant's 5DFR was checked for accuracy (e.g. portions of foods and beverages consumed, cooking methods, and branding information) in a one-on-one interview between the participant and a NZ Registered Dietitian. This data was then entered into and processed (energy, macro- and micronutrient nutrient analysis) within the dietary software analysis package, FoodWorks 10 (FoodWorks Professional 10; Xyris Software, Australia; New Zealand Food Composition Database). FoodWorks 10 was appropriate as it utilises NZ and Australia specific databases. NZ FOODFiles 2016 was the preferred database, and if the reported food item could not be found there, AusFoods 2017 and AusBrands 2017 (based on the Australian food composition databases AUSNUT 2011-13, developed by Food Standards Australia New Zealand) databases were used. Researchers independently entered the 5DFR into FoodWorks 10 and a second researcher checked data entry to confirm reliability. Where appropriate, all food and drink were entered in FoodWorks 10 as the cooked portion consumed. If only raw weights were provided, raw weights were converted to cooked weight. Cooking factors were used from McCance and Widdowson's (Roe et al., 2015), to allow for water and nutrient losses during the cooking process. To ensure consistency of data entry, a dietary data dictionary and standard operating procedure were developed and used throughout.

Nutrient intake estimates were only derived from food and beverages, intake from dietary supplements were excluded (with the exception of supplements that provided energy e.g. sports protein drink). The nutrient list for analysis included: Total energy (in kJ/day), total carbohydrate, starch, sugar, total dietary fibre, protein, total fat, saturated fat, monounsaturated fat, polyunsaturated fat. The following micronutrients were also analysed due to their importance for women's health; iron, B_{12}, iodine and calcium (Bartley et al., 2005). Total energy intake was calculated by summing energy contribution from all macronutrients including total dietary fibre, and alcohol (all in g/day). The percentage of energy intake from all macronutrients was calculated. Energy, macro- and micronutrient intakes were all reported

in standard units per/day. Energy intakes between 2100 kJ/day and 14600 kJ/day were considered plausible for valid completion of the 5DFR (Willett, 2012). Data was also checked for plausibility, where those who had an unrealistic high or low energy intake, were asked to clarify their intake (Ministry of Health New Zealand, 2011). After reviewing exported data, implausible data were interpreted as misreporting and excluded from further analysis.

A calibrated, Harpenden stadiometer was used to measure height and calibrated electronic scales (Sauter platform scale E1200, GmbH, Germany) were used to measure fasted body weight. Weight and height were used to calculate BMI (kg/m^2). Waist circumference was measured following ISAK protocol (Stewart A, 2011). Body composition (total body and visceral fat %) was assessed using dual-energy x-ray absorptiometry (DXA) (Hologic QDR Discovery A, Hologic Inc with APEX V. 3.2 software) (Kindleysides et al., 2019).

Between 7:30am and 9:00am, trained phlebotomists took blood samples (30ml) from fasted participants. Whole blood was stored immediately at −80° C in ethylenediaminetetraacetic acid (EDTA) vacutainers (Becton Dickinson). The remainder of the samples were processed within 1-hour of collection. Aliquots of plasma and serum were immediately stored at −80° C in 1.5 mL microcentrifuge tubes (Eppendorf® safe-lock PCR clean tubes, Hamburg, Germany) and cryovials (Cryo.S Greiner Bio-One, GmbH) (Kindleysides et al., 2019).

The Cobas e411 analyser (Hitachi High Technologies Corporation, Tokyo, Japan) was used to analyse serum levels of insulin, using the electrochemiluminescence immunoassay (ECLIA) method (Roche Diagnostics, Mannheim, Germany). The inter-assay coefficients of variation (%CV) for plasma glucose and insulin were 0.7 and 0.5%, respectively. Glycated haemoglobin (HbA1c) levels were measured from EDTA whole blood, by turbidimetric inhibition immunoassay (Roche Diagnostic, Mannheim, Germany) on a Hitachi c311 autoanalyser (Hitachi High Technologies Corporation, Tokyo, Japan). The inter-assay %CV for HbA1c was 1.0%.

NZ Deprivation Index 2013 (NZDep2013) (Atkinson J, 2014) was used as a measure of socioeconomic status; a score of 1 represents the areas "least deprived" and 10 "most deprived".

6.3 Statistical analysis

IBM SPSS software for Windows version 24.0 (SPSS Inc, Chicago, IL) was used for all statistical analysis. Statistical significance was tested at $p<0.05$. Histograms and Kolmogorov-Smirnov tests were used to confirm normality of data. Fasting plasma insulin was

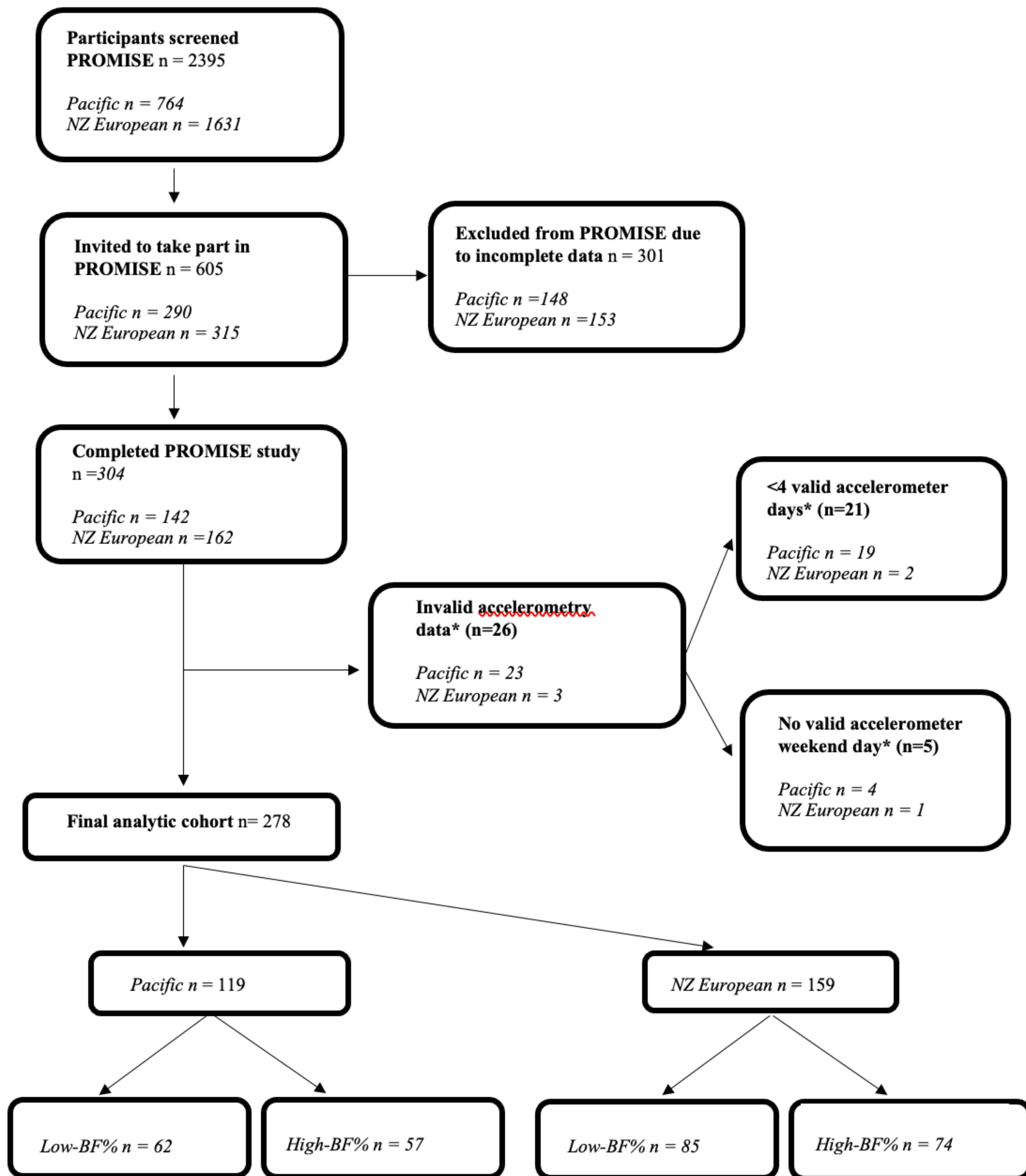
logarithmically transformed (*ln*) to ensure a normal distribution and reported as geometric means and geometric standard deviations (GSD). All other variables were reported as means and standard deviations (SD) to summarise all continuous data. Categorical variables are reported as frequencies (%). One-way ANOVA, with Bonferroni *post hoc* correction, was used to measure differences between ethnic and BF% groups. Logistic regression analysis was used to assess associations between nutrient intake and PA. New Zealand European women were significantly older ($p<0.05$) and less deprived ($p<0.05$) compared to Pacific women and considering age and deprivation are negatively associated with metabolic health (Ministry of Health New Zealand, 2021), all subsequent analysis was carried out controlling for age and deprivation index (NZDep2013). Also, NZE low-BF% group had significantly higher ($p<0.05$) sleep quality scores (PSQI) compared to all other groups. Considering poor sleep has been associated with higher BF% and reduced metabolic health, and it may lead to increased desire for energy dense foods and reduced desire for PA (Garaulet et al., 2011, Sullivan, 2016), all further analysis was controlled for sleep quality (PSQI).

Analyses were conducted separately for NZE and Pacific participants, as well as for both groups combined. For combined (NZE and Pacific) analyses, adjustments for ethnicity were also made.

6.4 Results

A total of 119 Pacific and 159 NZE women had valid data and were included in analysis (Figure 6.2). Table 6.1 provides the participant characteristics of NZE and Pacific women, stratified by body composition (high- and low-BF%). Participants in the low-BF% groups had a significantly lower body weight (Pacific low- 73.5 kg and high-BF% 99.8 kg and NZE low- 63.1 kg - and high-BF% 94.04 kg) compared to women in the high-BF% groups ($p<0.05$). Pacific women in the low-BF% group also had a significantly higher body weight and BMI compared to NZE women in the low-BF% group $p<0.05$) (Pacific low- 25.8 kg/m^2 and high-BF% 35.6 kg/m^2 and NZE low- 22.5 kg/m^2 and high-BF% 33.7 kg/m^2). Pacific and NZE women in the high-BF% had a total BF% close to 40% (39.5% and 41.2% respectively) and there was no statistical difference between the two ethnic groups. Mean plasma glucose was higher among Pacific women with a low-BF% ($p<0.05$) than for NZE with a low-BF%. There was no difference in mean glucose levels when comparing Pacific high and low-BF% groups nor when comparing high-BF% groups between ethnicities. However, glucose was significantly higher for NZE (5.52mmol.L) with a high-BF% when compared to their low-BF% counterparts, but was also the highest for all groups. Interestingly, Pacific women in both

BF% groups had significantly higher HbA1c (Pacific low- 32.2 mmol/mol and high-BF% 34.2 mmol/mol and NZE low- 30.4 mmol/mol and high-BF% 31.6 mmol/mol) ($p<0.05$) and insulin (Pacific low- 72.5 pmol/l and high-BF% 123 pmol/l and NZE low- 40.7 pmol/l and high-BF% 79.8 pmol/l) ($p<0.05$) when compared to NZE women. Both Pacific and NZE women with high-BF% had significantly higher HbA1c and insulin when compared to their low-BF% counterparts.



Legend
Pacific; women who identify as Pacific, NZ European ; women who identify as NZ European; *Participants were excluded from analyses if they did not wear the accelerometer for >12 h on >4 days including one weekend day. More in-depth information can be found elsewhere (Kindleysides et al., 2019)

Figure 6.2 Flowchart of participants included in the study

Table 6.1 Participant characteristics for Pacific and NZ European women by body fat % group

Characteristic	Pacific		NZ European	
	<35% BF Mean (SD)	≥35% BF Mean (SD)	<35% BF Mean (SD)	≥35% BF Mean (SD)
n	62	57	85	74
BMI (kg/m^2)	25.8 (3.9)†^	35.6 (6.0)	22.5 (2.1)	33.7 (3.8)
Underweight n (%)	0 (0)	0 (0)	1 (1)	0 (0)
Normal weight n (%)	30 (48)†^	2 (4)	73 (86)†	3 (4)
Overweight n (%)	23 (37)†^	5 (9)	9 (11)	3 (4)
Obese n (%)	9 (15)†^	50 (88)	2 (2)†	68 (92)
Age (years)	25 (7)^	25 (6)^	30 (7)†	33 (7)
Anthropometry				
Weight (kg)	73.5 (11.8)†^	99.8 (18.0)	63.1 (7.62)†	94.4 (12.3)
Height (cm)	168 (6.61)	168 (7.33)	167 (5.61)	167 (7.05)
Total BF% (%)	30.2 (3.09)†^	39.5 (3.41)	27.8 (4.46)†	41.2 (3.78)
Visceral fat (%)	27.2 (5.59)†^	39.6 (4.94)	22.2 (5.91)	39.7 (5.15)
Waist circumference	79.6 (7.95)†^	98.5 (13.1)	73.2 (5.75)†	98.0 (9.92)
Sleep quality				
PSQI global score	6 (3)	6 (3)	5 (2)†	7 (3)
Socioeconomic				
NZDep2013	7(3)^	8(2)^	4(2)	5(2)
Metabolic health markers				
Plasma glucose (mmol.L)	5.31 (0.47)^	5.48 (0.51)	5.12 (0.31)†	5.52 (0.46)
HbA1c (mmol.mol)	32.2 (1.98)†^	34.2 (2.93)^	30.4 (2.19)†	31.6 (2.81)
Plasma insulin (pmol/l)#	72.5 (1.74)†^	123 (1.93)^	40.7 (1.51)†	79.8 (1.61)

Values are presented as mean (standard deviation) unless otherwise specified; #= geometric mean (standard deviation of geometric mean); † $p< 0.05$ for test comparing body fat groups within ethnic group. ^$p< 0.05$ for test comparing between ethnic groups within body fat group; BMI, Body mass index; underweight (BMI < 18.5), normal (BMI ≥ 18.5 and < 25.0), overweight (BMI ≥ 25.0 and < 30) and obesity (BMI ≥ 30.0). HbA1c, fasting blood glycated hemoglobin. NZDep2013, NZDep2013 index of socioeconomic deprivation. BF, body fat; BMI, body mass index; PSQI, Pittsburgh sleep quality questionnaire; NZ, New Zealand

All groups' mean accelerometer wear times were >15 hours per day, and did not differ between ethnic or BF% groups (Table 6.2). Therefore, we did not adjust for accelerometer wear time in our statistical analysis. Pacific women in the low-BF% group engaged in significantly less total PA when compared to NZE women in the low-BF% group (p<0.05), however there was no difference between ethnic groups in total PA when comparing women in the high-BF% group. All groups spent less than 3% of the 24-hr day in MVPA. Pacific low- and high-BF% groups spent significantly less time in MVPA compared to their respective NZE BF% groups (Pacific high- 19.17 min/day, and low-BF%: 23.84 min/day and NZE high- 30.41 min/day and low-BF% (39.10 min/day) and NZE low-BF% spent significantly more time in MVPA compared to their high-BF% group counterparts. On average less than half Pacific women were meeting the PA guidelines (high-BF%; 39% and low-BF%; 47%) whereas just over 80% of NZE

women with low-BF% and 65% of NZE women in the high-BF% groups were meeting the PA guidelines. There were no significant difference in % of participants meeting the PA guidelines when comparing BF% group within each ethnic group.

Table 6.2 also provides the nutrient intake of NZE and Pacific women by BF% group. There was no significant difference in average TE intake between Pacific and NZE women or BF% groups. All groups consumed an average energy intake between 8308 and 9054 kJ/day. Energy intake per kg body weight did not differ between ethnic groups. Women in the low-BF% groups had significantly higher TE per kg body weight compared to their high-BF% counterparts (Pacific low-125 kJ/kg body weight and high-BF% 91 kJ/kg body weight and NZE low- 133 kJ/kg body weight and high-BF% 89 kJ/kg body weight).

On average Pacific women consumed significantly more carbohydrate compared to NZE women, although all groups consumed carbohydrate diets well below the acceptable macronutrient distribution range (AMDR). Within each ethnic group there was no difference in mean total carbohydrate intake (g/day) when comparing BF% groups. Average macronutrient distributions showed energy from carbohydrates fell below the AMDR of 45-65% of TE intake for the majority of NZE (36.9% low-BF% and 38.8% high-BF%) and Pacific women (43.0% low-BF% and 42.8% high-BF%). No women were consuming more than the carbohydrate AMDR (>65% TE). Mean sugar intake did not differ between BF% groups, however Pacific women's mean total sugar intake (added plus free sugars) was significantly higher than NZE women ($p<0.05$). Pacific women's mean daily starch intake was also significantly higher than NZE women but mean daily starch intake (g/day) did not differ between BF% groups. There was no difference in fibre intake between Pacific women in the high and low-BF% groups (19.8 and 19.2 g/day respectively), whereas NZE women in the low-BF% group had significantly higher fibre intake (26.2g, $p<0.05$) compared to NZE women in the high-BF% group (22.1g). There was no difference in fibre intake between Pacific and NZE women in the high-BF% group (19.2 and 22.1 g/day respectively). Only the NZE low-BF% group's mean fibre intake was above the recommended daily intake (RDI) of ≥25g/day, on average the other three groups were not meeting this recommendation.

There was no difference in mean protein intake (g/day) between all four groups, however as a percentage of TE Pacific low-BF% consumed significantly more protein compared to NZE women with a low-BF% (16.0% vs 17.7% respectively ($p<0.05$)). Energy from protein fell within the AMDR of 15-25% for all four groups, although all close to the lower range. Pacific

low-BF% group consumed less percentage of TE intake from protein compared to NZE low-BF% group; there was no difference between Pacific and NZE women in the high-BF% groups.

There was no difference in all four groups' mean total, saturated, mono- and poly-unsaturated fat intake (g/day). However, energy from fat was well above the AMDR of 20-35% for all four groups (Pacific low- 38.3% and high-BF% 38.7% and NZE low-39.9% and high-BF% 39.8%). Only a quarter of women in all groups met the AMDR for fat, with the remaining participants overconsuming and no participants under consuming. All groups also consumed at least 14.6% of TE from saturated fat, which is well above the recommended 8-10% (National Health and Medical Research Council, 2006).

There was no difference between Pacific and NZE women in mean iron intake for either high- or low-BF% groups. New Zealand European low-BF% groups mean iron intake (mg/day) was significantly higher than their high-BF% counterparts ($p<0.05$). There were no significant differences between all four groups mean vitamin B_{12}, and iodine intake. All four groups were consuming below the RDI for iron (18 mg/day) and calcium (1000 mg/day). Pacific high-BF% women were consuming the least calcium (254 mg/day), significantly less than Pacific low-BF% group (681 mg/day) and NZE high-BF% (875 mg/day) ($p<0.05$). Only the Pacific low-BF% group were meeting the RDI of 150 ug/day for iodine (173 ug/day) however there was no statistically significant difference in iodine between all four groups. All four groups were meeting the RDI for vitamin B_{12} (2.4 ug/day) and there was no significant difference between groups.

Table 6.2 Physical activity and dietary nutrient intake for Pacific and NZ European women by body fat % group

		Pacific		NZ European	
	Reference range	<35% BF	≥35% BF	<35% BF	≥35% BF
n		62	57	85	74
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Accelerometery					
Valid wear time per day (min/day)		948.6 (68.1)	953 (82.9)	930 (56.3)	930 (58.6)
MVPA (min/day)		23.8 (16.3)^	19.2 (15.1)^	39.1 (18.4)†	30.4 (19.2)
Total daily PA (cpm)		585 (168)^	596 (117)	718 (180)	652 (210)
Meet PA guidelines n (%)		29 (47)^	22 (39)^	69 (81)	48 (65)
Daily nutrient intake					
Energy (kJ/day)		9054 (2766)	8913 (2534)	8335 (1699)	8308 (1836)
Energy (kJ/kg body weight)		125 (37)†	91 (28)†	133 (29)†	89 (20)
CHO (g/day)		229 (77.8)^	225 (73.0)^	181 (54.6)	188 (56.2)
CHO %‡	45–65% TE*	43.0 (6.5)^	42.8 (6.1)^	36.9 (8.4)	38.9 (9.0)
Participants meeting CHO AMDR* n (%)		24 (39)^	23 (40)	14 (16)	20 (27)
Participants above CHO AMDR* n (%)		0 (0)	0 (0)	0 (0)	0 (0)
Participants below CHO AMDR* n (%)		38 (61)^	34 (60)	71 (84)	53 (72)
Sugars¥ (g)		94.8 (38.9)^	91.9 (42.3)^	77.8 (26.5)	82.6 (30.6)
Sugars¥ %‡(g)		17.8 (5.0)	17.5 (5.5)	16.0 (4.9)	17.2 (6.1)
Starch (g)		134 (47.5)^	133 (43.3)^	103 (40.2)	105 (34.0)
Starch %‡		25.2 (4.2)^	25.4 (4.6)^	20.8 (6.5)	21.7 (5.8)
Fibre (g)	≥25g/day	19.8 (7.4)^	19.2 (7.0)	26.2 (9.4)†	22.1 (6.8)
Protein (g)		84.5 (28.1)	86.2 (25.3)	85.3 (18.7)	85.0 (19.0)
Protein %‡	15–25% TE*	16.0 (3.4)^	16.7 (3.4)	17.7 (3.6)	17.6 (3.3)
Participants meeting protein AMDR* n		36 (58)	36 (63)	65 (76)	57 (77)
Participants above Protein AMDR* n (%)		3 (5)	1 (2)	2 (2)	1 (1)
Participants below Protein AMDR* n (%)		23 (37)	20 (35)	18 (21)	16 (22)
Total fat (g/day)		93.6 (30.7)	93.8 (30.6)	90.4 (27.2)	100 (30.9)
Total fat %‡	20–35% TE*	38.3 (4.9)	38.7 (4.8)	39.9 (7.7)	39.8 (7.9)

		Pacific		NZ European	
	Reference range	<35% BF	≥35% BF	<35% BF	≥35% BF
Participants meeting fat AMDR* n (%)		14 (23)	14 (25)	22 (26)	17 (23)
Participants above fat AMDR* n (%)		48 (77)	43 (75)	63 (74)	57 (77)
Participants below fat AMDR* n (%)		0 (0)	0 (0)	0 (0)	0 (0)
Saturated fat (g)		36.2 (13.6)	36.9 (13.4)	33.1 (12.2)	36.6 (14.9)
Saturated fat %‡	8-10% TE	14.7(2.5)	15.2 (2.8)	14.6 (4.0)	15.9 (4.1)
Monounsaturated fat (g)		34.9 (11.9)	35.3 (12.6)	33.7 (10.4)	32.8 (11.4)
Monounsaturated fat %‡		14.3 (2.3)	14.6 (2.8)	14.9 (3.4)	14.4 (3.4)
Polyunsaturated fat (g)		12.7 (4.6)	11.8 (4.8)	14.0 (6.2)	12.2 (5.8)
Polyunsaturated fat %‡		5.3 (1.5)^	4.9 (1.7)	6.2 (1.9)†	5.3 (1.9)
Iron (mg)	18 mg/day	11.9 (4.2)	11.6 (4.0)	12.9 (3.8)†	11.0 (3.1)
Vitamin B_{12} (ug)	2.4 ug/day	3.88 (1.6)	4.52 (2.11)	3.50 (1.56)	3.97 (2.18)
Iodine (ug)	150 ug/day	173 (552)	90.7 (42.5)	119 (98.9)	122 (162)
Calcium (mg)	1000 mg/day	681 (257)^	254 (250)^	892 (286)	875 (281)

† $p < 0.05$ for test comparing BF% groups within ethnic group; ^$p < 0.05$ for test comparing between ethnic groups within BF% group. PA, physical activity. MVPA, moderate to vigorous physical activity. *AMDR, Acceptable macronutrient distribution range (National Health and Medical Research Council, 2006). (National Health and Medical Research Council, 2006) reference ranges. BF, body fat. CHO, carbohydrate. ‡ Percentage energy from macronutrients calculated as % of total energy intake. ¥ includes all free and added sugars

Table 6.3 Odds of meeting physical activity guidelines when in quartile one of nutrient intake compared to the higher three quartiles

Dietary nutrient quartiles	Pacific	NZE	All
n	115	157	272
	OR/expβ (95% CI)	OR/expβ (95% CI)	OR/expβ (95% CI)
Energy (kJ)	0.99 (0.72, 1.37)	0.94 (0.67, 1.33)	0.96 (0.76, 1.21)
Energy (kJ/Kg body weight)	1.20 (0.84, 1.71)	0.74 (0.46, 1.18)	0.97 (0.74, 1.28)
CHO (g)	1.07 (0.77, 1.49)	1.28 (0.90, 1.81)	1.16 (0.91, 1.48)
% CHO‡	0.96 (0.63, 1.44)	1.38 (0.98, 1.94)	1.20 (0.93, 1.55)
Fibre (g)	0.79 (0.55, 1.14)	0.66 (0.46, 0.94)*	0.72 (0.55, 0.92)**
Sugars¥ (g)	0.93 (0.66, 1.30)	1.01 (0.72, 1.42)	0.98 (0.77, 1.25)
% Sugars‡	1.06 (0.74, 1.52)	1.17 (0.83, 1.63)	1.14 (0.90, 1.45)
Starch (g)	0.94 (0.66, 1.32)	1.37 (0.97, 1.95)	1.12 (0.88, 1.42)
% Starch‡	1.38 (0.91, 2.04)	1.26 (0.90, 1.76)	1.31 (1.02, 1.68)*
Protein (g)	1.05 (0.76, 1.44)	0.80 (0.56, 1.14)	0.92 (0.73, 1.16)
% Protein‡	1.18 (0.82, 1.70)	0.77 (0.55, 1.08)	0.94 (0.73, 1.20)
Total fat (g)	1.01 (0.77, 1.50)	0.79 (0.57, 1.11)	0.85 (0.67, 1.08)
% Total fat‡	1.02 (0.64, 1.54)	0.80 (0.55, 1.16)	0.86 (0.64, 1.14)
Saturated fat (g)	1.01 (0.73, 1.50)	0.87 (0.63, 1.21)	0.91 (0.72, 1.15)
% Saturated fat‡	0.91 (0.64, 1.30)	0.89 (0.64, 1.24)	0.98 (0.78, 1.25)
Monounsaturated fat (g)	0.91 (0.64, 1.29)	0.81 (0.59, 1.11)	0.84 (0.66, 1.06)
% Monounsaturated fat‡	0.95 (0.82, 1.09)	0.88 (0.64, 1.22)	0.87 (0.69, 1.11)
Polyunsaturated fat (g)	0.71 (0.50, 1.01)	1.05 (0.80, 1.38)	0.89 (0.71, 1.11)
% Polyunsaturated fat‡	0.67 (0.47, 0.96)*	0.86 (0.61, 1.21)	0.76 (0.59, 0.97)*
Iron (mg)	0.96 (0.68, 1.34)	0.92 (0.71, 1.22)	0.93 (0.76, 1.14)
Vitamin B_{12} (ug)	0.97 (0.67, 1.39)	0.92 (0.66, 1.28)	0.94 (0.74, 1.19)
Iodine (ug)	1.26 (0.90, 1.77)	0.79 (0.55, 1.13)	1.01 (0.80, 1.28)
Calcium (mg)	0.96 (0.67, 1.37)	0.87 (0.612, 1.24)	0.92 (0.72, 1.17)

NZ, New Zealand; PA, physical activity; MVPA, moderate to vigorous physical activity.
Model adjusted for age, NZDep2013 and sleep quality (global PSQI score); **p*<0.05, ***p*< 0.01, ****p*<0.001; †Meets PA guidelines = participates in ≥150 minutes of moderate intensity activity per week (Ministry of Health New Zealand, 2020) . Reference group = meeting the PA guidelines; Comparison group = not meeting PA guidelines). ¥ includes all free and added sugars. NZDep2013, NZ Deprivation Index 2013 ((Atkinson J, 2014). BF, body fat. CHO, carbohydrate. ʿAMDR, acceptable macronutrient distribution range (National Health and Medical Research Council, 2006). ‡ Percentage energy from macronutrients calculated as % of total energy intake.

Table 6.3 provides the odds of meeting the PA guidelines for participants in the lowest quartile of nutrient intake compared to the top three quartiles. Among NZ European women the odds of meeting the PA guidelines was 34% (*p*=0.021) lower among women who were in the lowest quartile of fibre intake compared to the top three quartiles. Among all women the odds of meeting the PA guidelines was 28% (*p*=0.008) lower among women who were in the lowest

quartile of fibre intake compared to the top three quartiles. Among all women the odds of meeting the PA guidelines was 31% (p=0.038) higher among women who were in the lowest quartile of starch (as a % of total energy intake) intake compared to the top three quartiles. There was a decreased odds of meeting the PA guidelines among women who were in the lowest quartile of polyunsaturated fat intake, particularly Pacific women, when measured as a % of total energy intake (all women OR 0.76, p=0.027 and Pacific women OR 0.67, p=0.030). Micronutrient dietary intakes (iron, vitamin B_{12} and iodine) were not significantly associated with meeting the PA guidelines. There were no further significant associations between macronutrient intake and meeting the PA guidelines.

6.5 Discussion

This study aimed to investigate whether meeting nutrient intake is associated with meeting the PA guidelines, in Pacific and NZE women, with low- (<35%) or high-BF% (≥35%). The findings of this study, indicate that among both Pacific and NZE women, there are minimal associations between dietary nutrient intake and meeting the PA guidelines. However, an interesting finding was the decreased odds of meeting the PA guidelines among women who were in the lowest quartile of fibre intake compared to the top three quartiles (NZE women OR 0.66, p=0.021 and all women OR 0.72, p=0.008), as previous findings from the PROMISE study found dietary fibre to be an indicator of diet quality (Renall, 2020). While some studies suggest that active people tend to eat healthier diets (Elder and Roberts, 2007), there are also a lot of discrepancies found in the literature examining the correlations between these health behaviours. Sport-involved males were found to have a higher mean daily energy intake (kcal/day) when compared to non-sport-involved males, however similar to this study's findings, no difference was found in the female group (Croll et al., 2006). Croll et al. (2006) also found no difference in mean total fat, protein and iron between sport-involved females and non-sport-involved females. They did find a significant difference in CHO intake as a percentage of TE between women participating in power team sports compared to non-sport-involved females. However this difference was only 1% therefore unlikely to have any clinical significance. Whilst the current studies found no association between calcium intake and meeting the PA guidelines, Croll et al. (2006) reported mean calcium to be significantly lower for non−sport-involved females than sport-involved females. However similar to the current study mean calcium intake in both groups was under the recommended daily intake for their population (1,300 mg). A study in African-American girls also found TE intake was not significantly associated with PA (Jago et al., 2004), however after controlling for household

income, material possessions, field centre, and total caloric consumption (BMI was found to be a non-significant confounder therefore not included in the models), PA (accelerometer counts per minute, minutes of MVPA, and mean METS per minute) was negatively associated with the percent of calories consumed from total fat (Jago et al., 2004). Although the current study found no association between meeting the PA and fat intake, among Pacific women, meeting the PA guidelines was associated with higher polyunsaturated fat intake than their counterparts who did not meet the PA guidelines. Unfortunately, Jago et al. (2004) did not further investigate the different types of fat (saturated and mono- and polyunsaturated) therefore we cannot compare to this finding.

Results of the current study also revealed differences between ethnic and BF% groups in both nutrient intake and PA that are interesting to discuss in relation to metabolic health. Firstly, we acknowledge, cross-sectional designs are susceptible to reverse causality. As PA and/or diet behaviour may change because of obesity therefore caution must be taken when interpreting these results. Furthermore, in the present study, nutrient profile rather than food or dietary patterns was assessed, therefore we cannot say what foods the nutrients are coming from (e.g., total sugars: there is a substantial difference between the nutrient density of fruit that contains naturally occurring sugars and a muesli bar with added sugar). We do not want to oversimplify a complex system and acknowledge nutrients are only one of the attributes of our diet that are relevant to health. Therefore, when interpreting these results, we can only provide insight into why the nutritional composition of a diet may be beneficial for metabolic health. Whilst no differences in dietary energy intake were found between Pacific and NZE women or BF% group, Pacific women's dietary intake differs from NZE, with significantly ($p<0.05$) higher carbohydrate intake contributing to TE intake. Firstly, although Pacific women had higher carbohydrate intake when compared to NZE women, when comparing to the AMDR for carbohydrate (45–65%), both ethnic groups did not consume large amounts of carbohydrate. According to the AMDRs Pacific women were consuming adequate carbohydrate whilst NZE women seem to be consuming more of a low carbohydrate high fat diet. When breaking carbohydrates down into its constituents this difference in carbohydrate intake was from starch rather than sugars. As a percentage of TE intake Pacific women's mean starch intake was higher than NZE women whereas there was no difference in sugar intake as a percentage of TE intake between ethnic groups. Further, fibre intake was particularly low, only mean intake of the NZE women in the low-BF% groups was above the recommended daily intake (RDI) of 25g/day. This is in agreement with Metcalf et al (2008), who also found that compared to NZE,

Pacific women had lower intakes of fibre and higher intakes of starch, when expressed in relation to total energy intake. Mean sugar intakes were above 15% of total energy for all four groups. Whilst the WHO recommends intake of free sugars to be <10% of total energy intake, in this study only total sugar intake was assessed therefore unfortunately it cannot be compared to this guideline. Carbohydrate intake, specifically increased sugar and low fibre, can be a key contributor to T2D (Afshin et al., 2019), as are low levels of PA (World Health Organisation, 2017). Pacific women and NZE women with high-BF% had significantly higher HbA1c and fasting insulin concentrations when compared to NZE women with low-BF%. High-BF% is known to mediate metabolic outcomes such as T2D (Guh et al., 2009), and Pacific women are a high metabolic disease risk population with 9.5% diagnosed with T2D (Ministry of Health New Zealand, 2021). Therefore, considering carbohydrate foods are the main dietary source of fibre but also of sugar which can be a contributor to T2D (Afshin et al., 2019), it would be interesting to determine the main food sources of each nutrient, in particular fibre, starch and sugar. For example, if main sources of starch are low fibre ultra-processed foods such as white breads and sweetened breakfast cereals, this would be more concerning for metabolic health compared to non-processed higher fibre starchy foods such as kūmara and potatoes. Similarly, if the main food source of sugars is from foods with naturally occurring sugars that also contain fibre such as fruit this would be less concerning for metabolic health compared to foods with added/free sugars (but no fibre) such confectionary. It is important to note that participants in this study were a convenience sample of women living in Auckland NZ, which does limit the generalisability of the findings. Therefore, future studies should explore these relationships in other populations such as in men and in different age, BMI and ethnic groups within different regions.

The present study also supports evidence that differences in metabolic disease risk between Pacific and NZE women are associated with differences in level of deprivation. As mentioned previous findings from the PROMISE study found dietary fibre to be an indicator of diet quality (Renall, 2020). In this study, the NZE low-BF% group were participating in the most PA and consuming a diet with the most fibre. They also had the lowest HbA1c, fasting plasma insulin and glucose concentrations, lowest total BF% and visceral BF%, indicating their metabolic health risk may be lower than Pacific women and NZE women with a high-BF%. New Zealand European women in the low-BF% group also had a lower level of deprivation compared to all other groups. In comparison the highest deprivation group was Pacific women with a high-BF%. Women in this group also had the highest HbA1c and fasting plasma insulin

concentrations, participated in the least MVPA and had the lowest percent of women meeting the PA guidelines (39%) and alongside NZE high-BF% had the highest total and visceral BF%. All these results suggest they are a higher metabolic disease risk group. Studies have found that people who live in more deprived communities have reduced food security, for example poorer access to fruit and vegetables (Eagle et al., 2012, Cummins and Macintyre, 2006), which could lead to a poorer diet quality. The 2008/09 NZ National Nutrition Survey highlighted that Pacific women were nearly three times more likely to live in a household that had low food security compared to non-Pacific women (Ministry of Health New Zealand, 2012). A systematic review found lower quality diets, e.g. high in saturated fat, added sugar, and sodium, as well as energy dense foods were cheaper on a per-kilojoule basis when compared to higher quality diets (comprised of beneficial nutrients including protein, fibre, vitamins, and minerals) (Darmon and Drewnowski, 2015). They also found lower income groups tend to select cheaper and more energy dense diets, often lacking fruit and vegetables (Darmon and Drewnowski, 2015). Specifically in NZ, living in the most deprived areas decreases the likelihood of consuming the recommended daily servings of fruit and vegetables by 33%, in comparison to individuals living in the least deprived areas (Ministry of Health New Zealand, 2021). Furthermore, living in deprived communities can also cause isolation from PA promoting environments (e.g. safe places to exercise, safe side walks, bike paths and parks) (Friel et al., 2007). Adding to this, higher level of deprivation in early years of development, has been linked to reduced development of parts of the brain that contribute to regulation and control of behaviours and thoughts (Marteau and Hall, 2013). This includes level of cognitive control over participation in PA and diet, reiterating that people who live in more deprived areas are in a more disadvantaged position, with less resources to combat obesity and poor metabolic health (Li et al., 2014, Friel et al., 2007). Thus, deprivation should always be considered when developing or refining any public health initiative focused on diet and PA.

The objective measurement of PA is a strength to this study. However, when wearing an accelerometer, some individuals do change their PA behaviours, especially on the first day of monitoring (Baumann et al., 2018). Data from the first and last days of monitoring were removed from analysis to account for this. In addition, it is recognised that women are more likely to underreport dietary intake when compared to men, and under reporting is also more prevalent among individuals with obesity or who are overweight when compared to lean subjects (Archundia Herrera and Chan, 2018). We minimised this limitation by removing all participants reporting an energy intake less than 2100 kJ/day (Willett, 2012) and reviewed

5DFR's for plausible intakes. Also, we chose to use food records estimated with household measures as they cause less disruption to participants normal eating patterns and food habits than the weighing of food; thus, likely capturing a better reflection of habitual intake as opposed to a reflection of actual intake during the record-keeping period alone.

Time spent in PA and total amount of PA are only one aspect of PA. Physical activity also encompasses type and intensity of activity. O'Brien et. al (2019) have shown the type of PA Pacific and NZE women choose to engage in differs (O'Brien et al., 2019). Whilst both ethic groups reported high participation in walking, Pacific women also reported music and dance among their top four activities and NZE mainly took part in "gym-type activities"(O'Brien et al., 2019).

Together with nutrients our diet is about food and dietary patterns, including meals and ways of eating such as social and cultural aspects of our diet. Combined they have important implications for understanding how diet can improve health and prevent disease (Tapsell et al., 2016). It would be interesting for future research to investigate the relationship between foods consumed or dietary patterns and PA in this population. This would help improve our understanding of the relationship between food choice and PA within this high (Pacific) and moderate (NZE) metabolic disease risk population.

6.6 Conclusions

There was a lack of association between nutrient intake and meeting the PA guidelines observed in the present study. Considering the current study and previous research have shown dietary and PA patterns differ between ethnic groups, in particular Pacific and NZE women, the next logical step for research would be to focus on individual foods, food groups (particularly carbohydrates and fats) and PA patterns (including types of PA), specific to each ethnic group, and their combined relationship with body composition and metabolic health. This research also highlights the need for vast improvements to be made to ensure deprivation is considered in the promotion of healthful diet and PA pattern.

6.7 References

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Chapter 7: Is objectively measured physical activity and sedentary behaviour associated with the gut microbiota among Pacific and New Zealand European women?

Abstract

Aim: Investigate whether physical activity (PA) and sedentary behaviour (SB) are associated with broad taxonomic composition (family level) and α-diversity of the gut microbiota, in a population of Pacific and NZ European (NZE) women.

Methods: Pacific (n=119) and NZE (n=159) women, aged 18-45 years were recruited according to self-reported normal or obese body mass index (BMI). PA and SB were assessed using eight-day accelerometery. Body composition was assessed using BMI and BF% (whole body dual-energy x-ray absorptiometry), and the latter was used to stratify women as low (<35%) or high (≥35%) BF%. DNA was extracted from faecal samples and the metagenomic sequences associated with the microbiota were analysed using MetaPhlAN and QIIME2 software. Multivariate regression analysis, adjusted for potential confounders was conducted to explore the association between microbiota composition, PA and SB.

Results: Among NZE women, every one SD increase in total PA (197 cpm/day) was associated with 36.3% higher relative abundance of *Erysipelotrichaceae* (*p*=0.031) and 37.9% lower relative abundance of *Verrucomicrobiaceae* (*p*=0.029). Additionally, every one SD increase in SB (1.45 hours/day) was associated with a 28% lower relative abundance of *Erysipelotrichaceae* (*p*=0.030). After controlling for all potential confounders, for every 1 SD increase in total PA NZE women were 1.62 times more likely to be characterised by enterotype 1 and 41.6% less likely to be characterised by enterotype 3. Furthermore, for every 1 SD increase in SB, NZE women were 39.4% less likely to be characterised by enterotype 1 and 1.829 times more likely to be characterised by enterotype 3. The only significant finding among Pacific women was that for every 1 SD increase in NZE women's total PA was associated with 23.1% higher *Firmicutes:Bacteroidetes* ratio (*p*=0.031), whereas among Pacific women, every 1 SD increase in MVPA was associated with 22.8% lower (p=0.034) *Firmicutes:Bacteroidetes* ratio.

Conclusion: Increased total PA and reduced SB may be positively associated with the relative abundance of bacteria that are capable of producing gut microbiota metabolites (e.g. SCFA's). Associations between PA and gut microbiota composition may differ between ethnic groups.

In order to clearly interpret these results more research is needed to determine the mechanism driving associations between PA, SB and the gut microbiota.

7.1 Introduction

Healthy habits, including diet, sleep and physical activity (PA) are the foundations for overall health and wellbeing. Increasingly research is associating these lifestyle factors with the gut microbiota (Clark and Mach, 2016, Chandrakumaran et al., 2016, Cronin et al., 2021). The gut microbiota is a complex, ecological community of micro-organisms residing in the gastrointestinal tract. In normal healthy conditions the gut microbiota plays an important role in the digestion and absorption of nutrients, maintenance of the intestinal lining and immunity (Ursell et al., 2012, Devaraj et al., 2013). Factors such as genetics, mode of delivery at birth, method of infant feeding, use of certain medications, age and certain lifestyle choices (diet, PA and sleep) have all been shown to contribute to the composition of the gut microbiota (Wen and Duffy, 2017). Compared with research on diet, research on the relationship between sleep or PA and gut microbiota is less developed, therefore the focus of this research was on associations between PA and the gut microbiota composition, whilst ensuring the potential confounding factors of diet and sleep are controlled for.

A major function of the gut microbiota is to ferment non-digestible substrates like dietary fibres, therefore, diet is currently considered the main extrinsic factor that can alter the microbiome (Cronin et al., 2021). Diets rich in, plant-derived foods and fibre, which are recommended in an optimal diet to promote human health (Willett et al., 2019), have been associated with increased microbial diversity (García-Vega et al., 2020). Further, bacterial fermentation of carbohydrates (e.g. resistant starch, simple sugars, and polysaccharides) produces short-chain fatty acids (SCFA) including acetate, propionate, and butyrate (Topping and Clifton, 2001). These SCFAs have regulatory functions in anti-inflammatory and immune response, lipid, cholesterol and glucose metabolism as well as gut barrier integrity (Fart et al., 2020). Evidence is emerging to suggest sleep behaviour may play a role in various aspects of the gut microbiome namely abundance of certain taxa, diversity and function (Potter et al., 2016) (Thaiss et al., 2014). Research has demonstrated negative effects on the gut microbiota when the circadian rhythm is disrupted; intestinal microbiota exhibits diurnal oscillations in rats and this can be misaligned with a society-enforced circadian rhythm (Zarrinpar et al., 2014).

Physical activity (PA) performed at levels recommended by the World Health Organisation (WHO) (i.e., accumulation of ≥ 30 minutes of moderate or greater intensity activity on ≥ 5 days per week, or 150 minutes of MVPA per week (World Health Organisation, 2020, Ministry of Health New Zealand, 2020, U.S. Department of Health and Human Services, 2018)), is associated with a range of beneficial health effects, including a reduced risk of obesity, cardiometabolic disease and T2D. Some of the health benefits of PA may be derived from interactions through the gut microbiota (Cerdá et al., 2016). A number of mechanisms have been proposed explaining the association between PA and microbiota composition, however there is no common consensus (Chandrakumaran et al., 2016). Nevertheless, it is important to discuss some potential mechanisms for the interaction. Altered gut motility or activity is one mechanism by which exercise may influence the gut microbiome. Physical activity reduces gut transit time (Song et al., 2012, Shahar et al., 2020) and changes in gut transit may have effects on intestinal pH, biofilm formation, and availability of nutrients to microbes. Furthermore, lactate is also released into the blood during PA, which could alter intestinal pH if any of this lactate is secreted into the gut lumen (Brooks, 1991). Alterations in the pH of the gut may influence the bacteria able to grow and thrive. Although commonly referred to, the mechanism by which PA modulates SCFAs remains speculative (Carey and Montag, 2021).

When compared to sedentary controls, athletes appear to have higher relative abundance of bacterial taxa with anti-inflammatory properties and capacity to synthetise SCFAs in their faecal microbiota (Ticinesi et al., 2019, Barton et al., 2018), which in turn may result in improved the gut health such as maintenance of intestinal barrier integrity, protection against inflammation, or *lipid, cholesterol and glucose metabolism (Fart et al., 2020).*Furthermore, increased cardiorespiratory fitness (VO_{2max}) has been positively correlated with increased bacterial diversity and butyrate-producing bacteria such as the *Erysipelotrichaceae* family (Estaki et al., 2016).

A healthy microbiota can be described regarding increased richness and diversity as well as compositional (bacterial phyla, genera, family taxa) and functional features (metabolic properties). Microbial dysbiosis such as reduction in microbial diversity, including the loss of beneficial bacteria such as *Bacterioides* and butyrate producing bacteria (e.g. *Faecalibacterium*) have been identified as a contributing factor in the development of a range of metabolic diseases including obesity and type 2 diabetes (T2D) (Hartstra et al., 2015, Bäckhed et al., 2004, El-Jurdi et al., 2017, Turnbaugh et al., 2009). Microbial dysbiosis can be determined using a range of measures, including but not limited to, diversity (α or β), species

richness or microbial abundances such as relative abundance (%) at various taxonomic levels (e.g family, genera or species), and alterations in the ratio between the dominant phyla *Firmicutes* and *Bacteroidetes* (*Firmicutes:Bacteroidetes*). Decreased microbiota diversity and richness, and a higher ratio *Firmicutes:Bacteroidetes,* have been associated with obesity, T2D and altered blood glucose (Magne et al., 2020, Letchumanan et al., 2022). Although lower ratio *Firmicutes:Bacteroidetes* has been associated with good health, an optimal *Firmicutes:Bacteroidetes* ratio for human health has not been defined (Magne et al., 2020). The abundance of *Firmicutes* in the gut microbiota of healthy individuals been shown to vary between 11% to 95% and that of Bacteroidetes between 0.6% to 86.6% (Magne et al., 2020).

Most human studies investigating PA and the gut microbiota recruit elite athletes or physically fit individuals. However, the majority of the world population is physically inactive, with less than one third (31%) (Hallal et al., 2012) meeting WHO recommendations for PA of ≥30 minutes of moderate (or greater) intensity activity on at least five days of the week (U.S. Department of Health and Human Services, 2018, Ministry of Health New Zealand, 2020). Therefore, to better understand the associations observed between PA and the gut microbiota in the general public, especially high metabolic disease risk populations, more research is needed. There should be a particular focus on the contribution of meeting current WHO PA guidelines to gut microbiota composition (Shahar et al., 2020). Further, despite variations in the gut microbiota composition of healthy individuals from different ethnic groups (Deschasaux et al., 2018), most research in this area has been carried out in European populations (Clarke et al., 2014a, Bressa et al., 2017, Barton et al., 2018). Thus, it is important that different, including minority, ethnic groups are included in future research, as their unique gut microbiota profile may respond differently to PA when compared to European populations.

The aim of this study was therefore to explore the possibility that PA (MVPA, total PA, sedentary behaviour and/or meeting PA guidelines *versus* not meeting PA guidelines) are associated with broad taxonomic composition (family level) and α-diversity of the gut microbiota intestinal bacteria, that could drive beneficial metabolic changes to human hosts. Associations will be explored among two NZ population groups with different metabolic disease risk (Pacific and NZ European (NZE) women) and different body fat (BF) profiles (normal and obese). We hypothesise that increased time spent in PA, reduced time spent in sedentary behaviour and meeting the PA guidelines will be associated with increased α-diversity and relative abundance of health promoting bacteria (family level) in the human gut.

7.2 Materials and Methods

Participants were part in the cross-sectional PRedictors linking Obesity and the gut MIcrobiomE (PROMISE) study (Kindleysides et al., 2019). Briefly, post-menarche and pre-menopausal (as defined by regular menstrual cycles over the last year) Pacific and NZE women, who were aged 18-45 years and in self-reported general good health were invited to participate in the study. Participants were screened using self-reported height and weight to calculate BMI (kg/m^2). Women with a BMI within the predefined normal or obese BMI ranges (BMI ≥18.5 to <25.0 kg/m^2 and ≥30.0 kg/m^2, respectively), were invited to participate.

A calibrated Harpenden stadiometer was used to measure height to the nearest millimetre. Body weight was measured in a fasted state, on calibrated electronic scales (Sauter platform scale E1200, GmbH, Germany) measuring to the nearest 0.01 kg. Repeated measures of height and weight were taken following ISAK protocols (Stewart A, 2011). Weight and height were then used to calculate BMI (kg/m^2) to use for statistical analysis. Total BF% was assessed using dual-energy x-ray absorptiometry (DXA) (Hologic QDR Discovery A, Hologic Inc with APEX V. 3.2 software) by accredited researchers (Australian and NZ Bone Mineral Society clinical densitometry accreditation) (Kindleysides et al., 2019).

People with the same BMI can have heterogeneity of BF and metabolic disease risk factors (Oliveros et al., 2014, Kramer et al., 2013, Dickey et al., 1998). Therefore, participants were subsequently classified into two groups based on measured BF%: low-BF% (<35%) and high-BF% (≥35%). The American Association of Clinical Endocrinologists and American College of Endocrinology guidelines (obesity in women >35%) were used to classify BF% cut-offs (Dickey et al., 1998, Oliveros et al., 2014, Jo and Mainous III, 2018). Individuals were excluded from the study if they had been diagnosed with chronic illness (e.g., T2D and cardiovascular disease), or were pregnant or lactating.

Participants attended two clinic visits, 11-14 days apart and they provided written informed consent prior to participation. The study was approved by the Health and Disability Ethics Committees Ethics committee (HDEC, reference: 16/STH/32) and the trial was prospectively registered at anzctr.org.au (ACTRN12618000432213). Demographic and health information was gathered using a standardised face-to-face interview with a trained researcher (e.g., address, personal/household income, and medication use).

Physical activity, diet and sleep

A w-GT3X tri-axial accelerometer (Actigraph LLC, 2016), worn on the nondominant hip, was used to measure PA. An Acti-Watch (Micro Motionlogger®) worn on the non-dominant wrist

was used to measure sleep (Ward et al., 2005) (Ancoli-Israel et al., 2003). Both devices were set to record with an epoch length of one minute. For the following eight days participants wore both devices continuously (24-hour protocol), except when participating in water activities such as swimming or bathing/showering. Participants also completed a daily PA and sleep diary during this period, recording any time the accelerometers were removed and put back on as well as sleep onset; and end times for any sleep ≥10 minutes duration. To record sleep onset and wake time, participants pressed the Acti-Watch "event" button when they went to bed and turned off their light to sleep, and when they woke up. Acti-Life® software (version 6.13.3, Actigraph) was used to process the w-GT3X accelerometer data. Data from the first and last days (days of partial wear) of monitoring was removed, due to incomplete data and to allow for subject reactivity on the first day of wearing. Data sets were in a 24-h midnight-midnight format. Sleep diaries were used to identify sleep periods and sleep periods were confirmed using the ActiWatch accelerometer data. Only epochs that occurred during waking hours were used for analysis of PA and SB.

Non-wear time for the wGT3X was defined as ≥ 60 consecutive minutes of zero epoch counts, allowing for two minutes of counts between zero and 100. Data was considered valid if the accelerometer was worn for ≥12 hours/day, on ≥ four days, this included one weekend day (Tudor-Locke et al., 2012). Mean time (min/day) spent in MVPA (≥2020 counts per minute (cpm)), light intensity PA (LPA) (100 - 2019 cpm) and sedentary behaviour (SB) (0 – 99 cpm), was calculated from valid data (Troiano et al., 2008).

Participants completed the validated Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989), which respectively assesses sleep quality over a one-month period. The PSQI was completed online (SurveyMonkey Inc, 2017). It covers seven areas in which sleep problems occur and provides a global sleep quality score, ranging from 0 to 21, lower scores suggest a healthier sleep quality (Buysse et al., 1989).

A prospective, five-day food record (5DFR), was used to collect dietary intake data. Food record days were pre-allocated and non-consecutive, including at least one weekend day (Gibson, 2005). To improve adherence and to reduce participant burden, estimated, as opposed to weighed, food records were used (Gibson, 2005, Livingstone and Black, 2003). Verbal training for filling out the food record and estimating food and beverage portions was provided. To help estimate portion sizes, participants were given a standardised food portion booklet, and standard household food measures. Participants also watched a 15-minute food record instruction and demonstration video (developed by Massey University researchers).

Upon return, participants were interviewed by a NZ Registered Dietitian to check food record accuracy (e.g. portions of foods and beverages consumed, cooking methods, and branding information) Dietary data were entered and processed (energy, macro- and micronutrient nutrient analysis) using an electronic food processing system (Foodworks 10; Xyris Software, Australia). Foodworks 10 utilises the NZ specific databases (NZ FOODFiles 2016). However, if the food or beverage could not be found in this database, AusFoods 2017 and AusBrands 2017 (based on the Australian food composition databases AUSNUT 2011-13, developed by Food Standards Australia New Zealand), were used. Independently, two researchers entered the 5DFR into Foodworks 10 and to confirm reliability a second researcher checked data entry. All food and drink were entered as the cooked portion consumed (where appropriate)(Roe et al., 2015). When only raw weights were provided, these were converted to cooked weight using cooking factors to allow for water and nutrient losses. A dietary data dictionary and standard operating procedure were developed to ensure consistent data entry. The nutrient list for analysis included; total energy (in kJ/day), total carbohydrate, starch, sugar, total dietary fibre, protein, total fat, saturated fat, monounsaturated fat, polyunsaturated fat (all in g/day). To calculate total energy intake, energy contribution from all macronutrients including total dietary fibre, and alcohol (all in g) was summed. Energy and macronutrient intakes were reported in standard units per/day. Energy intakes between 2100 kJ/day and 14600 kJ/day were considered as valid completion of the 5DFR in the PROMISE study population group (Willett, 2012).

NZ Deprivation Index 2013 (NZDep2013) (Atkinson J, 2014) was used as a proxy of socioeconomic status. NZDep2013 was derived from geographical area of residence, which combines census data relating to home ownership, housing, qualifications, income, employment, access to transport, communications, and family structure. NZDep2013 score ranges from one which represents the areas "least deprived" to 10 "most deprived".

Gut microbiota

Participants collected faecal samples within the three days following removal of their accelerometer devices and completion of their 5DFR. Duplicate samples from the same faeces were collected. Participants placed the provided kidney dish in the toilet to catch the faeces. They then used the scoop that was inside the lid of two small, screw top containers provided to collect two walnut-sized faecal samples. Participants then filled two larger provided containers with 2 cm of water and placed one of the smaller scoop containers inside each of the larger containers, so the small containers were surrounded by water. The date and time of faecal

sample collection was recorded on both large containers. The faecal samples were then immediately placed into their household freezer (-20° C) until they were returned to the clinic. An insulated chilly bag and an ice-pack, was provided for transport of the faecal samples to the clinic. Upon arrival to the research clinic a researcher, without delay, stored the faecal samples in the -80° C freezer until analysis. Faecal samples were transported by World Courier© using dry ice to maintain temperature, to the University of Otago, Microbiology department for microbiota analysis

Gut microbiota analysis

DNA extraction and sequencing:. DNA was extracted from 250 mg faeces according to the kit protocol provided by the manufacturer (PowerSoil DNA isolation kit, Mo Bio, Carlsbad, CA, USA), with the following modification: faecal samples were suspended in 1mL of TN150 buffer (containing 10 mM TRIS-CL pH 8.0, 150 mM NaCl). The suspension was centrifuged at 14,600×g (3 min, 5°C) and then suspended in 700 µl solution from the PowerBead Tubes. The suspension was added back to the PowerBead Tubes and the standard protocol followed. DNA was eluted in 100 µl of elution buffer (70°C) and stored at -80°C. To check quality and quantity of genomic DNA, a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) were used. Then the cleaned DNA was sent to New Zealand Genomics Ltd. (NZGL) to carry out shotgun metagenome sequencing.

NZGL prepared 384 Thruplex DNA libraries and carried out 2×125 bp paired-end sequencing across 24 lanes on an Illumina HiSeq 2500 instrument (Illumina, San Diego, CA, USA). To sequence libraries, a minimum of six HiSeq lanes were used. To test for library preparation and sequence run bias, multiple libraries were prepared for several samples. An average of 13,150,561 (range 7,6940,894-17,081,755) reads were recovered for each sample. For quality control BBDuk (https://sourceforge.net/projects/bbmap/) was used to trim adapters, remove low quality reads and remove reads <100 bp after trimming. KneadData (http://huttenhower.sph.harvard.edu/kneaddata) was used as quality control to remove human genome reads (i.e., DNA) from bacterial reads, implementing the hg19 database. Sequence data used in this study will be deposited with the short-read archive (SRA).

7.3 Statistical and bioinformatic analysis

Previous work by the PROMISE study team carried out bioinformatic analysis on the microbiota data. Microbiota taxonomic profiles were created from DNA sequences using MetaPhlan 2.0 (version 2.6.0) according to default parameters (Segata et al., 2012). QIIME2

(version 2018.8 (Bolyen et al., 2018) (https://qiime2.org/) was used to analyse microbiota composition and α-diversity, using converted output tables from MetaPhlan 2.0. PERMDISP was used to measure group dispersion (Anderson, 2006). The composition of the participant's microbiota was characterised in terms of enterotypes (Renall, 2020). Three enterotypes were identified Table 7.1. Enterotypes were predicted using R statistical software following the approach described in Arumugum et al. (Arumugam et al., 2011, Renall, 2020).

Table 7.1 Description of enterotypes identified by PROMISE study

Enterotype	**Characterised by abundance of**	**Predominately found in population**
Enterotype 1	*Eubacterium rectale and Faecalibacterium prausnitzii* (butyrate-producing bacterial species)	both Pacific and NZE women
Enterotype 2	*Bifidobacterium adolescents, Bifidobacterium bifidum, Lactobacillus ruminis* (lactic acid-producing bacterial species)	Pacific women
Enterotype 3	*Subdoligranulum species, Akkermansia muciniphila, Ruminococcus bromii, and Methanobrevibacter smithii*	NZE women

(Renall, 2020)

Statistical analysis was carried out using IBM SPSS software for Windows version 24.0 (SPSS Inc, Chicago, IL). Normality of data was tested using histograms and Kolmogorov-Smirnov tests. *Erysipelotrichaceae, Rikenellaceae, Verrucomicrobiaceae, Veillonellaceae* and *Firmicutes:Bacteroidetes* ratio were logarithmically transformed (ln) to ensure a normal distribution.

The 10 bacterial families with the highest relative abundance were included in descriptive analyses and as outcomes in regression analyses, the mean relative abundance of these 10 families totalled >90% in both Pacific and NZE women. Alpha-diversity is a measure of the diversity of the microbiome within a given individual. This study used the Shannon index and Pielou's Evenness as measures of α-diversity (richness/ evenness). For each participant the *Firmicutes:Bacteriodetes* ratio was calculated by dividing the relative abundance of the phyla *Firmicutes* by the relative abundance of *Bacteriodetes*.

Means and standard deviations (SD) were used to summarise all continuous data, except variables that were logarithmically transformed, which were presented as geometric means and geometric standard deviations (GSD). Frequencies (%) were reported for categorical variables. Differences between ethnic and BF% groups were measured with one-way ANOVA, with Bonferroni post hoc correction. Separate multiple linear regression models were used to assess associations between PA and SB variables and bacterial relative abundance and α-diversity.

Logistic regression models were used to assess associations between PA and SB variables and presence/absence of enterotypes 1-3. PA and SB variables included total PA (cpm), light PA (min/day), MVPA (min/day) and meeting vs not meeting the PA guidelines and SB (hr/day). All independent variables were assessed for collinearity by assessing tolerance and the variance inflation factor (VIF). Sedentary behaviour and light PA were found to be highly correlated (VIF = 11.7, tolerance = 0.085); therefore, these variables were not included in the same regression models, instead associations with each of these two variables were assessed in separate regression models.

Regression analyses were conducted separately for NZE and Pacific participants. Results are presented for both unadjusted (referred to as model 1 in the Tables) and adjusted (for age, deprivation, BF% group, sleep quality (PSQI), dietary fibre intake (models 2 and 3) and either MVPA, light PA or SB, where appropriate (models 4 and 5). Regression coefficients (β) obtained from log-transformed data represent relative differences and were expressed as a ratio (by using e^{β}). For all PA and SB variables we expressed the difference in gut microbiota per 1 SD increase of PA or SB. Associations with *p*-values <0.05 were considered statistically significant.

7.4 Results

Dietary, PA, microbiota and general study population characteristics are presented in Table 7.2. A total of 351 women took part in the wider PROMISE study, with 304 participants completing all aspects of the study (Kindleysides et al., 2019). Of these women, 278 provided valid accelerometer data, and were eligible to take part in this study: 119 Pacific (43%) and 159 NZE women (Table 2). Pacific women were significantly younger than NZE women with high and low BF% both had a mean age 25 years. NZE low and high-BF% 30 and 33 years respectively ($p<0.05$). There were no differences in total BF% between Pacific and NZE women in the high BF% group. Pacific women in the low BF% group had significantly higher total BF% compared to their NZE counterparts ($p<0.05$). Compared to NZE women with a high BF%, NZE women with a low BF% reported significantly better sleep quality, as measured by the PSQI ($p<0.05$). Pacific women were significantly more deprived than NZE women ($p<0.05$). Less than half of the Pacific women were meeting the PA guidelines (high-BF%; 22% and low-BF%; 47%) compared with 81% (low-BF%) and 65% (high-BF%) of NZE women. All groups consumed an average energy intake between 8308 and 9054 kJ/day with all participants falling within the 2100 kJ/day and 14600 kJ/day cut-offs (Willett, 2012).

and there was no significant difference in average total energy intake between all four groups. Pacific women consumed significantly more carbohydrate (g/day) than NZE women ($p<0.05$), but no difference when comparing BF% groups within each ethnic group. There was no difference in fibre intake between Pacific women in the high and low BF% groups (19.2 and 19.8 g/day respectively), whilst NZE women in the low-BF% group consumed significantly more fibre compared to the high-BF% group and Pacific women in the low BF% group ($p<0.05$). There was no difference in fibre intake between Pacific and NZE women in the high-BF% group (19.2 and 22.1 g/day respectively). Out of all the four groups, only the NZE low-BF% group's mean fibre intake was above the recommended daily intake (RDI) of ≥25g/day.

When investigating α-diversity (number of predicted species, Pielou's Eveness and Shannon index) the average number of predicted species ranged from 70 among NZE women with a high BF%, to 76 among Pacific women with a low BF%. There were no significant differences between all four groups when comparing number of predicted species. The microbiota of NZE women with a low BF% was characterised by significantly higher Pielou's evenness (0.68) (distribution of species present) when compared to their Pacific counterparts (0.71). There was no difference in Shannon index between groups ranging from 4.2 in both Pacific and NZE women with a high-BF% to 4.4 in NZE women with a low BF%. Pacific women with a high-BF% had a significantly higher *Firmicutes:Bacteroides* ratio when compared to all other groups ($p<0.05$). There was no significant difference in the *Firmicutes:Bacteroides* ratio between NZE women with a low- and high-BF%.

There was no difference between all four groups in relative abundance of *Ruminococcaceae, Lachnospiraceae*, *Eubacteriaceae, Coriobacteriaceae and Veillonellacea.* Pacific women had significantly higher relative abundance of *Bifidobacteriaceae* and *Rikenellaceae* compared to NZE women (*Bifidobacteriaceae*; Pacific low- and high-BF% and NZE low- and high-BF% respectively; 16.88%, 18.52%, 6.66% and 8.03% and *Rikenellaceae;* Pacific low- and high-BF% and NZE low- and high-BF% respectively; 1.38%, 0.73%, 2.72% and 1.79%). Relative abundance of *Erysipelotrichaceae* was significantly higher among Pacific women compared to NZE women and Pacific women with a low-BF% (0.25% vs 0.78% respectively) had significantly higher relative abundance compared to their high-BF% (0.32% vs 2.56% respectively) counterparts. Pacific versus NZE women with a high-BF% had a significantly lower relative abundance of *Verrucomicrobiaceae* (0.73% and 1.39% respectively). Relative

abundance of *Bacteroidaceae* was significantly lower among Pacific women with a high- versus low-BF% (3.56% vs 7.83%) and NZE women with a high BF% (7.44%).

Enterotype 2 was predominately characterising Pacific women, and particularly significant among those with a high-BF% compared to a low-BF% ($p<0.05$); Pacific women high- and low-BF%, n = 35 and n = 21 respectively, compared to NZE women high- and low-BF% n = 6 and n = 4 respectively ($p<0.05$). Significantly more NZE women were characterised by enterotype 3 (n = 33 and n = 27, low- and high-BF% respectively), compared to Pacific women (n = 8 and n = 1, low- and high-BF% respectively) ($p<0.05$). There was no significant difference in percentage of Pacific and NZE women or women with a high or low-BF% who were characterised by enterotype 1.

Table 7.2 Demographic, microbiota, body composition and physical activity characteristics of Pacific and NZE women (n=278) by body fat % group

Characteristic	Pacific		NZE	
	<35% body fat Mean (SD)	**≥35% body fat Mean (SD)**	**<35% body fat Mean (SD)**	**≥35% body fat Mean (SD)**
N	62	57	85	74
Age (years)	25 (7)^	25 (6)^	30 (7)†	33 (7)
Anthropometry				
Weight (kg)	73.5 (11.8)†^	99.8 (18.0)	63.1 (7.62)†	94.4 (12.30)
Height (cm)	168 (6.61)	168 (7.33)	167 (5.61)	167 (7.05)
BMI (kg/m^2)	25.8 (3.9)†^	35.6 (6.0)	22.5 (2.1)	33.7 (3.8)
Underweight n (%)	0 (0)	0 (0)	1 (1)	0 (0)
Normal weight n (%)	30 (48)†^	2 (4)	73 (86)†	3 (4)
Overweight n (%)	23 (37)†^	5 (9)	9 (11)	3 (4)
Obese n (%)	9 (15)†^	50 (88)	2 (2)†	68 (92)
Total BF% (%)	30.2(3.09)†^	39.5(3.41)	27.8(4.46)†	41.2 (3.78)
Sleep quality				
PSQI global score	6 (3)	6 (3)	5 (2)†	7 (3)
Socioeconomic				
NZDep2013	7(3)^	8(2)^	4(2)	5(2)
Accelerometery				
Valid wear time per day (min/day)‡	949 (68.1)	953 (82.9)	930 (56.3)	929.84 (58.6)
MVPA (min/day)	23.8 ± 16.3^	19.2 (15.1)^	39.1 (18.4)†	30.4 (19.2)
Light physical activity (min/day)	310 (79.3)	344 (95.9)	328 ± 79.5	320 ± 86.0
Standardised total sedentary time« (hr/day)	10.4 (1.38)^	9.93 ± 1.59	9.69 ± 1.32	9.96 ± 1.58
Total physical activity (cpm)	585 (168)^	596 (177)	718 (180)	652 (210)
Meet PA guidelines n (%)	29 (47)^	22 (39)^	69 (81)	48 (65)
Daily nutrient intake				
Energy (kJ/day)	9054 (2766)	8913 (2534)	8335 (1699)	8308 (1836)
Total CHO (g/day)	228.8 (77.8)^	224.8 (73.0)^	181.0 (54.6)	188 (56.2)
• Total sugars¥ (g/day)	94.8 (38.9)^	91.9 (42.3)^	77.8 (26.5)	82.6 (30.6)
• Total starch (g/day)	133.9 (47.5)^	132.7 (43.3)^	103.0 (40.2)	105.0 (34.0)
Total dietary fibre (g/day)	19.8 (7.4)^	19.2 (7.0)	26.2 (9.4)†	22.1 (6.8)
Total protein (g/day)	84.5 (28.1)	86.2 (25.3)	85.3 (18.7)	85.0 (19.0)
Total fat (g/day)	93.6 (30.7)	93.8 (30.6)	90.4 (27.2)	100.0 (30.9)
Microbiota characteristics				
Alpha-diversity				
Predicted species abundance (n)	76 (12)	72 (12)	73 (9)	70 (9)
Pielou's Evenness	0.68 (0.05)^	0.67 (0.06)	0.71 (0.05)	0.69 (0.05)
Shannon index	4.3 (0.4)	4.2 (0.5)	4.4 (0.4)	4.2 (0.4)
Firmicutes: Bacteroidetes ratio#	5.5 (2.5)†	14.6 (3.0)^	5.8 (2.7)	8.2 (3.7)
Relative abundance (%)				
Ruminococcaceae	26.41 (10.68)	25.13 (12.87)	29.11 (9.29)	26.34 (10.41)
Lachnospiraceae	11.92 (5.13)	13.41 (7.87)	17.47 (6.50)	19.25 (10.13)

Characteristic	Pacific		NZE	
	<35% body fat Mean (SD)	**≥35% body fat Mean (SD)**	**<35% body fat Mean (SD)**	**≥35% body fat Mean (SD)**
Eubacteriaceae	13.85 (9.34)	14.75 (9.75)	15.24 (8.65)	18.95 (11.08)
Bifidobacteriaceae	16.88 (13.46)^	18.52 (13.62)^	6.66 (8.28)	8.03 (8.03)
Bacteroidaceae	7.83 (8.11)†	3.56 (4.09)^	8.86 (6.87)	7.44 (7.70)
Erysipelotrichaceae#	0.78 (8.67)†^	2.56 (6.11)^	0.25 (6.30)	0.32 (4.95)
Rikenellaceae#	1.38 (5.42)^	0.73 (5.31)^	2.72 (3.00)	1.79 (4.85)
Coriobacteriaceae#	2.12 (2.55)	3.03 (1.91)	1.87 (2.28)	2.03 (2.73)
Verrucomicrobiaceae#	0.39 (7.69)	0.73 (5.31)^	1.25 (11.02)	1.39 (10.80)
Veillonellaceae#	1.25 (8.00)	0.44 (12.56)	0.30 (15.03)	0.13 (16.44)
Enterotype (n present (%))				
Enterotype 1	33 (53)	21 (37)	48 (56)	41 (55)
Enterotype 2	21 (34)†^	35 (61)^	4 (5)	6 (8)
Enterotype 3	8 (13)^	1 (2)^	33 (39)	27 (36)

Values are presented as Mean ± standard deviation unless otherwise specified; #= geometric mean ± standard deviation of geometric mean); † *p*< 0.05 for test comparing body fat groups within ethnic group. ^*p*< 0.05 for test comparing between ethnic groups within body fat group; BMI, Body mass index; underweight (BMI < 18.5), normal (BMI ≥ 18.5 and < 25.0), overweight (BMI ≥ 25.0 and < 30) and obesity (BMI ≥ 30.0). NZDep2013, NZDep2013 index of socioeconomic deprivation; PSQI = Pittsburgh sleep quality index - score ranges from 0 to 21, lower scores suggest a healthier sleep quality; Pacific n=5 with missing deprivation data, NZE n=1 with missing deprivation data. Meet PA guidelines = engages in ≥150 minutes of moderate intensity activity per week (Ministry of Health New Zealand, 2020), MVPA = moderate to vigorous physical activity,. ¥ includes all free and added sugars; ‡Excluding sleep time; «Standardised to a 16 hour day

There was no difference in relative abundance of bacterial species between women who met the PA guidelines and those who did not meet the PA guidelines (Table 7.3). Among Pacific women, not meeting the PA guidelines was associated with a 54.2% (p=0.026) higher *Firmicutes:Bacteroidetes* ratio compared to Pacific women who did meet the PA guidelines (Table 7.3). NZE women who did not meet the PA guidelines Pielou's Evenness (-0.025, p=0.013) and Shannon Index (-0.155, p=0.031) were significantly lower compared to NZE women who did meet the PA guidelines (Table 7.3, model 3).

Table 7.3 Association between meeting or not meeting the physical activity guidelines and microbiota family relative abundance (%) and alpha-diversity

Microbiota characteristic	**Pacific**			**NZE**		
	Model 1	**Model 2**	**Model 3**	**Model 1**	**Model 2**	**Model 3**
N	119	117	115	159	158	157
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Relative abundance (%)						
Ruminococcaceae	-1.46 (-5.78, 2.86)	-0.818 (-5.20, 3.57)	0.147 (-4.28, 4.58)	-1.35 (-4.87, 2.17)	-0.712 (-4.30, 2.87)	-0.620 (-4.28, 3.34)
Lachnospiraceae	-0.139 (-2.57, 2.29)	0.151 (-2.25, 2.55)	-0.094 (-2.54, 2.35)	-2.03 (-5.01, 0.95)	-2.32 (-5.35, 0.71)	-2.42 (-5.49, 0.639)
Eubacteriaceae	0.429 (-3.07, 3.93)	0.151 (-3.28, 3.58)	0.517 (-3.00, 4.04)	1.74 (-1.81, 5.29)	1.07 (-2.54, 4.68)	1.34 (-2.33, 5.01)
Bifidobacteriaceae	1.98 (-2.98, 6.94)	1.43 (-3.58, 6.45)	0.575 (-4.49, 5.63)	2.60 (-0.28, 5.48)	2.08 (-0.797, 4.96)	1.94 (-1.00, 4.89)
Bacteroidaceae	-2.00 (-4.48, 0.49)	-1.53 (-3.88, 0.822)	-1.38 (-3.79, 1.03)	-0.977 (-3.57, 1.61)	-0.866 (-3.50, 1.77)	-0.688 (-3.34, 1.97)
Coriobacteriaceae	0.578 (-0.220, 1.38)	0.504 (-0.301, 1.31)	0.415 (-0.393, 1.22)	0.241 (-0.596, 1.08)	0.134 (-0.726, 0.993)	0.132 (-0.748, 1.01)
	Ratio (95% CI)	Ratio (95% CI)	Ratio (95% CI)	Ratio (95% CI)	Ratio (95% CI)	Ratio (95% CI)
Erysipelotrichaceae†	1.19 (0.555, 2.56)	1.07 (0.500, 2.27)	0.919 (0.426, 1.98)	0.751 (0.405, 1.39)	0.747 (0.399, 1.40)	0.705 (0.373, 1.33)
Rikenellaceae†	0.774 (0.407, 1.47)	-0.721 (0.383, 1.36)	0.736 (0.385, 1.40)	0.796 (0.491, 1.29)	0.862 (0.525, 1.41)	0.797 (0.483, 1.31)
Verrucomicrobiaceae†	1.20 (0.393, 3.70)	1.22 (0.388, 3.82)	1.14 (3.03, 3.91)	2.10 (0.770, 5.73)	2.06 (0.742, 5.74)	1.70 (0.647, 4.45)
Veillonellaceae†	1.18 (0.456, 3.06)	1.09 (0.421, 2.84)	1.05 (0.391, 2.82)	0.558 (0.175, 1.79)	0.676 (0.202, 2.26)	0.683 (0.201, 2.32)
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
Alpha-diversity						
Predicted species	1.00 (-3.54, 5.54)	-0.143 (-4.30, 4.03)	-1.14 (-5.33, 3.06)	-0.722 (-3.99, 2.54)	-0.032 (-3.36, 3.30)	-0.244 (-3.51, 3.02)
Pielou's Evenness	0.006 (-0.016, 0.029)	0.002 (-0.019, 0.024)	0.001 (-0.003, 0.001)	-0.028 (-0.046, -0.009)**	-0.025 (-0.044, -0.006)*	-0.025 (-0.044, - [illegible]
Shannon index	0.048 (-0.129, 0.226)	0.010 (-0.157, 0.177)	-0.014 (-0.184, 0.156)	-0.181 (-0.316, -0.046)**	-0.154 (-0.292, -0.016)*	-0.155 (-0.296, - [illegible]
	Ratio (95% CI)	Ratio (95% CI)	Ratio (95% CI)	Ratio (95% CI)	Ratio (95% CI)	Ratio (95% CI)
Firmicutes:Bacteroidetes†	1.60 (1.07, 2.39)*	1.52 (1.05, 2.20)*	1.54 (1.05, 2.26)*	1.11 (0.734, 1.69)	1.05 (0.686, 1.60)	1.06 (0.687, 1.62)

Model 1: Unadjusted. Model 2: Adjusted for age, deprivation index, body fat % group. Model 3: Model 2 + also adjusted for sleep quality (PSQI global score), dietary fibre intake (g/day)

*P value <0.05, **P value ≤0.01, ***P value ≤0.001. † data has been log transformed (*ln*). Pacific, participants who identify as Pacific. NZE, participants who identify as New Zealand European.

β (95% CI) = Difference between meeting and not meeting PA guidelines (reference group = meeting the PA guidelines and comparison group = not meeting PA guidelines)
Regression coefficients (β) obtained from log-transformed data represent relative differences and were expressed as a ratio (by using e^{β}).

Among NZE women, every one SD increase in total PA (197 cpm/day) was associated with 36.3% higher relative abundance of *Erysipelotrichaceae* (Table 7.4, model 3, *p*=0.03) and 37.9% lower relative abundance of *Verrucomicrobiaceae* (Table 7.4, model 3, *p*=0.029).

Among Pacific women, total PA was not associated with any measure of α-diversity (Table 4). Among NZE women, every 1 SD increase in total PA, was associated with 0.009 higher Pielou's Evenness (Table 4 model 1, *p*=0.041). However, after controlling for BF% group, significant associations were no longer observed. Also after controlling for all potential confounders, every 1 SD increase in NZE women's total PA was associated with 23.1% higher *Firmicutes:Bacteroidetes* ratio (Table 4, model 3, *p*=0.031).

Table 7.4 Association between total physical activity (cpm/day) and microbiota family relative abundance (%) and alpha-diversity

Microbiota characteristic	Pacific			NZE		
	Model 1	**Model 2**	**Model 3**	**Model 1**	**Model 2**	**Model 3**
n	119	117	115	159	158	157
	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase
Relative abundance (%)						
Ruminococcaceae	1.22 (-1.24, 3.68)	1.33 (-1.16, 3.81)	1.05 (-1.45, 3.53)	0.266 (-1.29, 1.83)	0.227 (-1.37, 1.82)	0.128 (-1.51, 1.76)
Lachnospiraceae	-0.127 (-1.51, 1.26)	-0.153 (-1.53, 1.22)	-0.064 (-1.44, 1.32)	0.557 (-0.766, 1.88)	0.770 (-0.584, 2.13)	0.879 (-0.492, 2.25)
Eubacteriaceae	-0.378 (-1.49, 0.737)	-0.898 (-2.86, 1.06)	-1.02 (-3.00, 0.961)	0.935 (-0.635, 2.51)	1.21 (-0.386, 2.81)	1.21 (-0.419, 2.84)
Bifidobacteriaceae	-0.275 (-3.11, 2.56)	-0.545 (-3.42, 2.33)	-0.310 (-3.17, 2.54)	-0.754 (-2.04, 0.529)	-0.456 (-1.75, 0.833)	-0.374 (-1.70, 0.946)
Bacteroidaceae	0.406 (-1.03, 1.84)	0.713 (-0.636, 2.06)	0.678 (-0.685, 2.04)	-0.659 (-1.80, 0.485)	-0.895 (-2.06, 0.270)	-0.975 (-2.15, 0.202)
Coriobacteriaceae	-0.174 (-0.631, 0.284)	-0.246 (-0.707, 0.216)	-0.210 (-0.667, 0.247)	-0.035 (-0.406, 0.336)	-0.012 (-0.395, 0.371)	-0.011 (-0.404, 0.381)
	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase
Erysipelotrichaceae	0.894 (0.578, 1.38)	0.880 (0.572, 1.36)	0.918 (0.595, 1.42)	1.31 (0.997, 1.72)	1.34 (1.01, 1.77)*	1.36 (1.03, 1.81)*
Rikenellaceae	1.30 (0.902, 1.88)	1.35 (0.941, 1.92)	1.34 (0.932, 1.93)	1.21 (0.730, 2.01)	0.859 (0.690, 1.07)	0.875 (0.701, 1.09)
Verrucomicrobiaceae	0.656 (0.344, 1.25)	0.623 (0.313, 1.24)	1.62 (0.296, 1.28)	0.596 (0.382, 0.931)*	0.587 (0.374, -0.923)*	0.621 (0.406, 0.950)*
Veillonellaceae	1.24 (0.734, 2.10)	1.23 (0.720, 2.09)	1.24 (0.721, 2.15)	1.21 (0.730, 2.01)	1.16 (0.687, 1.97)	1.17 (0.688, 2.00)
	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase
Alpha-diversity						
Predicted species abundance	-0.718 (-3.32, 1.88)	-0.149 (-2.56, 2.26)	0.049 (-2.35, 2.44)	0.338 (-1.11, 1.79)	0.220 (-1.26, 1.71)	0.067 (-1.39, 1.53)
Pielou's Evenness	-0.007 (-0.019, 0.006)	-0.007 (-0.019, 0.006)	-0.006 (-0.019, 0.006)	0.009 (0.000, 0.017)*	0.007 (-0.002, 0.015)	0.007 (-0.002, 0.016)
Shannon index	-0.049 (-0.150, 0.052)	-0.044 (-0.140, 0.053)	-0.038 (-0.135, 0.058)	0.057 (-0.003, 0.188)	0.044 (-0.018, 0.106)	0.041 (-0.022, 0.105)

	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase
Firmicutes:Bacteroidetes	0.873 (0.692, 1.10)	0.834 (0.674, 1.03)	0.833 (0.670, 1.04)	1.17 (0.970, 1.40)	1.23 (1.02, 1.48)*	1.23 (1.02, 1.49)*

Model 1: Unadjusted. Model 2: Adjusted for age, deprivation index, body fat % group. Model 3: Model 2 + also adjusted for sleep quality (PSQI global score), dietary fibre intake (g/day)

*P value <0.05, **P value ≤0.01, ***P value ≤0.001. † data has been log transformed (*ln*). Pacific, participants who identify as Pacific. NZE, participants who identify as New Zealand European

Regression coefficients (β) represents one standard deviation higher total physical acitivity (197 cpm/day). Regression coefficients (β) obtained from log-transformed data represent relative differences and were expressed as a ratio (by using e^{β}).

Among Pacific but not NZE women, every one SD (19.2 min/day) increase in MVPA was associated with higher *Bacteroidaceae* relative abundance (1.53% Table 7.5, model 1, p=0.04). Every one SD increase in MVPA was also associated with higher *Verrucomicrobiaceae* relative abundance (0.607%, unadjusted, p=0.029) among NZE but not Pacific women. However, after adjusting for BF% group, both these associations were no longer statistically significant.

The unadjusted analysis in NZE women showed every one SD increase in MVPA was associated with significantly higher α-diversity; Pielou's Evenness (0.009, p=0.031) (Table 7.5, model 1), however, after controlling for BF% group, this association was no longer statistically significant (Table 7.5, model 2). Every 1 SD increase in MVPA was associated with 22.8% lower (Table 7.5 model 5, p=0.034) *Firmicutes*:*Bacteroidetes* ratio, among Pacific women. Unadjusted and adjusted results were highly comparable.

Table 7.5 Association between moderate to vigorous physical activity (MVPA; min/day) and microbiota family relative abundance (%) and alpha-diversity

Microbiota characteristic	**Pacific**					**NZE**				
	Model 1	**Model 2**	**Model 3**	**Model 4**	**Model 5**	**Model 1**	**Model 2**	**Model 3**	**Model 4**	**Model 5**
n	119	117	115	115	115	159	158	157	157	157
	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase
Relative abundance (%)										
Ruminococcaceae	1.59 (-0.991, 4.18)	1.27 (-1.40, 3.93)	0.893 (-1.79, 3.58)	0.317 (-2.58, 3.21)	0.289 (-2.45, 3.03)	0.678 (-0.878, 2.23)	1.27 (-1.40, 3.93)	0.200 (-1.48, 1.88)	0.068 (-1.71, 1.85)	0.063 (-1.64, 1.77)
Lachnospiraceae	0.107 (-1.35, 1.57)	-0.111 (-1.58, 1.35)	0.037 (-1.45, 1.52)	0.175 (-1.43, 1.78)	0.334 (-1.19, 1.85)	0.641 (-0.68, 1.96)	-0.111 (-1.58, 1.35)	1.06 (-0.350, 2.47)	1.03 (-0.46, 2.52)	1.03 (-0.404, 2.46)
Eubacteriaceae	-0.811 (-2.91, 1.29)	-0.708 (-2.80, 1.38)	-0.809 (-2.94, 1.32)	-0.476 (-2.78, 1.83)	-0.625 (-2.83, 1.58)	0.031 (-1.56, 1.59)	-0.708 (-2.798, 1.381)	0.453 (-1.24, 2.14)	0.077 (-1.70, 1.86)	0.298 (-1.41, 2.01)
Bifidobacteriaceae	-1.08 (-4.06, 1.90)	-0.883 (-2.17, 0.40)	-0.316 (-3.39, 2.75)	0.058 (-3.26, 3.38)	-0.004 (-3.18, 3.17)	-0.909 (-2.19, 0.37)	-0.620 (-3.68, 2.44)	-0.148 (-2.17, 0.541)	-0.886 (-2.32, 0.549)	-0.779 (-2.16, 0.599)
Bacteroidaceae	1.53 (0.048, 3.02)*	1.16 (-0.269, 2.59)	1.12 (-0.340, 2.57)	1.09 (-0.485, 2.67)	1.08 (-0.428, 2.59)	0.012 (-1.14, 1.16)	1.16 (-0.269, 2.59)	-0.220 (-1.44, 1.00)	0.068 (-1.22, 1.36)	-0.129 (-1.37, 1.11)
Coriobacteriaceae	-0.449 (-0.925, 0.028)	-0.390 (-0.879, 0.099)	-0.395 (-0.883, 0.092)	-0.403 (-0.931, 0.125)	-0.390 (-0.896, 0.115)	-0.330 (-0.696, 0.037)	-0.390 (-0.879, 0.099)	-0.315 (-0.717, 0.086)	-0.370 (-0.795, 0.054)	-0.331 (-0.739, 0.078)
	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase
Erysipelotrichaceae†	0.931 (0.587, 1.47)	1.067 (0.673, 1.69)	1.12 (0.705, 1.79)	1.29 (0.780, 2.12)	1.23 (0.763, 1.99)	1.15 (0.874, 1.51)	1.07 (0.744, 1.69)	1.23 (0.920, 1.65)	1.12 (0.818, 1.50)	1.17 (0.873, 1.57)
Rikenellaceae†	1.38 (0.947, 2.01)	1.42 (0.973, 2.07)	1.40 (0.953, 2.05)	1.32 (0.869, 2.01)	1.34 (0.898, 1.99)	1.04 (0.836, 1.28)	1.42 (0.973, 2.07)	1.03 (0.817, 1.30)	1.08 (0.850, 1.38)	1.05 (0.830, 1.32)
Verrucomicrobiaceae†	1.02 (0.507, 1.19)	0.931 (0.448, 1.93)	0.983 (0.449, 2.15)	1.12 (0.499, 2.50)	1.06 (0.482, 2.32)	0.607 (0.388, 0.948)*	0.931 (0.448, 1.93)	0.666 (0.424, 1.05)	0.731 (0.455, 1.18)	0.720 (0.457, 1.14)
Veillonellaceae†	0.815 (0.470, 1.42)	0.803 (2.18, 1.40)	0.818 (0.459, 1.46)	0.705 (0.376, 1.33)	0.736 (0.406, 1.34)	1.08 (0.647, 1.80)	0.803 (0.460, 1.40)	1.03 (0.589, 1.80)	1.00 (0.555, 1.80)	1.01 (0.572, 1.78)
	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase
Alpha-diversity										
Predicted species abundance	-0.389 (-3.12, 2.34)	0.167 (-2.36, 2.69)	0.593 (-1.94, 3.13)	0.498 (-2.22, 3.22)	0.600 (-2.01, 3.21)	0.062 (-1.38, 1.51)	-0.337 (-0.750, 0.076)	-0.497 (-1.99, 1.00)	-0.705 (-2.29, 0.875)	0.031 (-0.035, 0.097)

Microbiota characteristic	**Pacific**					**NZE**				
	Model 1	**Model 2**	**Model 3**	**Model 4**	**Model 5**	**Model 1**	**Model 2**	**Model 3**	**Model 4**	**Model 5**
n	119	117	115	115	115	159	158	157	157	157
Pielou's Evenness	0.000 (-0.014, 0.013)	0.001 (-0.012, 0.014)	0.002 (-0.011, 0.015)	0.005 (-0.009, 0.019)	0.004 (-0.010, 0.017)	0.009 (0.001, 0.017)*	0.008 (-0.001, 0.016)	0.008 (-0.001, 0.017)	0.006 (-0.004, 0.015)	0.007 (-0.002, 0.016)
Shannon index	-0.005 (-0.112, 0.101)	0.012 (-0.090, 0.113)	0.022 (-0.081, 0.124)	0.041 (-0.069, 0.150)	0.032 (-0.072, 0.137)	0.057 (-0.004, 0.117)	0.043 (-0.019, 0.104)	0.041 (-0.025, 0.106)	0.025 (-0.043, 0.094)	0.031 (-0.035, 0.097)
	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase
Firmicutes:Bacteroidete†	0.716 (0.564, 1.10) **	0.755 (0.604, -1.06)*	0.753 (0.598, -1.05)*	0.772 (0.601, -0.991)*	0.772 (0.608, 0.980)*	0.994 (0.827, 1.20)	1.04 (0.861, 1.26)	1.02 (0.841, 1.25)	0.963 (0.783, 1.18)	1.00 (0.821, 1.23)

Model 1: Unadjusted. Model 2: Adjusted for age, deprivation index, body fat % group. Model 3: Model 2 + also adjusted for sleep quality (PSQI global score), dietary fibre intake (g/day). Model 4: Model 3 + also adjusted for sedentary behaviour. Model 5: Model 3 + also adjusted for light physical activity (min/day).
*P value <0.05, **P value ≤0.01, ***P value ≤0.001. † data has been log transformed (*ln*). Pacific, participants who identify as Pacific. NZE, participants who identify as New Zealand European.
Regression coefficients (β) represents one standard deviation higher MVPA (19.19 min/day). Regression coefficients (β) obtained from log-transformed data represent relative differences and were expressed as a ratio (by using e^{β}).

SB was not associated with microbiota family relative abundance in Pacific women (Table 7.6). Among NZE women, every one SD increase in SB (1.45 hours/day) was associated with a 28% lower relative abundance of *Erysipelotrichaceae* (p=0.030); This association remained after controlling for BF% group, age, deprivation index, sleep quality, dietary fibre intake and MVPA.

Among Pacific women, SB was not associated with any measures of α-diversity (Table 7.6). Every one SD increase in NZE women's SB, was associated with a significantly lower Pielou's Evenness (-0.010, p=0.023) and Shannon index (-0.065, p=0.038) (Table 7.6 model 1), however after controlling for BF% group associations were no longer statistically significant (Table 7.6 models 2-4).

Table 7.6 Association between sedentary behaviour (hours/day) and microbiota family relative abundance (%) and alpha diversity by ethnicity

Microbiota characteristic	Pacific				NZE			
	Model 1	**Model 2**	**Model 3**	**Model 4**	**Model 1**	**Model 2**	**Model 3**	**Model 4**
n	119	117	115	115	159	158	157	157
	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase
Relative abundance (%)								
Ruminococcaceae	-1.13 (-3.21, 0.946)	-1.41 (-3.54, 0.717)	-1.32 (-3.43, 0.801)	-1.22 (-3.51, 1.07)	-0.265 (-1.82, 1.29)	-0.446 (-2.06, 1.17)	-0.419 (-2.05, 1.21)	-0.398 (-2.13, 1.33)
Lachnospiraceae	0.171 (-1.00, 1.34)	0.304 (-0.869, 1.48)	0.241 (-0.93, 1.42)	0.293 (-0.979, 1.57)	-0.257 (-1.58, 1.07)	-0.379 (-1.75, 1.00)	-0.404 (-1.78, 0.972)	-0.082 (-1.53, 1.37)
Eubacteriaceae	0.662 (-1.02, 2.35)	0.805 (-0.87, 2.48)	0.847 (-0.839, 2.53)	0.706 (-1.12, 2.53)	-1.14 (-2.70, 0.426)	-1.20 (-2.81, 0.417)	-1.16 (-2.79, 0.471)	-1.14 (-2.87, 0.592)
Bifidobacteriaceae	0.482 (-1.92, 2.88)	0.792 (-1.66, 3.25)	0.775 (-1.65, 3.20)	0.792 (-1.84, 3.42)	0.575 (-0.708, 1.86)	0.105 (-1.20, 1.41)	0.058 (-1.26, 1.38)	-0.218 (-1.61, 1.18)
Bacteroidaceae	0.164 (-1.05, 1.38)	-0.352 (-1.51, 0.805)	-0.374 (-1.54, 0.791)	-0.050 (-1.30, 1.20)	0.660 (-0.481, 1.80)	0.797 (-0.384, 1.98)	0.849 (-0.331, 2.03)	0.870 (-0.380, 2.12)
Coriobacteriaceae	0.036 (-0.352, 0.424)	0.125 (-0.270, 0.521)	0.104 (-0.287, 0.494)	-0.016 (-0.434, 0.403)	-0.052 (-0.422, 0.318)	-0.049 (-0.436, 0.339)	-0.050 (-0.443, 0.342)	-0.166 (-0.578, 0.246)
	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase
Erysipelotrichaceae†	1.15 (0.795, 1.66)	1.25 (0.864, 1.80)	1.24 (0.856, 1.79)	1.33 (0.897, 1.98)	0.698 (0.534, 0.913)**	0.708 (0.530, 0.929)*	0.698 (0.527, 0.924)*	0.720 (0.535, 0.969)*
Rikenellaceae†	0.856 (0.627, 1.17)	0.810 (0.594, 1.10)	0.815 (0.596, 1.11)	0.890 (0.641, 1.25)	1.12 (0.894, 1.37)	1.16 (0.927, 1.45)	1.14 (0.913, 1.43)	1.17 (0.841, 1.48)
Verrucomicrobiaceae†	1.44 (0.843, 2.44)	1.43 (0.800, 2.55)	1.46 (0.792, 2.69)	1.49 (0.789, 2.82)	1.52 (0.972, 2.40)	1.57 (0.985, 2.50)	1.46 (0.945, 2.26)	1.33 (0.841, 2.10)
Veillonellaceae †	0.856 (0.550, 1.33)	0.830 (0.527, 1.31)	0.831 (0.523, 1.32)	0.742 (0.448, 1.23)	0.901 (0.546, 1.49)	0.920 (0.543, 1.56)	0.914 (0.540, 1.55)	1.09 (0.525, 1.59)
	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase	Difference [β (95% CI)] per one SD increase
Alpha-diversity								

Microbiota characteristic	Pacific				NZE			
	Model 1	**Model 2**	**Model 3**	**Model 4**	**Model 1**	**Model 2**	**Model 3**	**Model 4**
n	119	117	115	115	159	158	157	157
Predicted species abundance	0.188 (-2.00, 2.38)	-0.356 (-2.41, 1.69)	-0.358 (-2.39, 1.68)	-0.218 (-2.40, 1.96)	-0.341 (-1.80, 1.12)	-0.392 (-1.91, 1.12)	-0.424 (-1.90, 1.05)	-0.644 (-2.20, 0.908)
Pielou's Evenness	0.006 (-0.005, 0.016)	0.006 (-0.005, 0.017)	0.006 (-0.005, 0.017)	0.007 (-0.004, 0.019)	-0.010 (-0.018, -0.001)*	-0.008 (-0.017, 0.001)	-0.008 (-0.017, 0.001)	-0.006 (-0.016, 0.003)
Shannon index	0.036 (-0.049, 0.121)	0.033 (-0.049, 0.115)	0.031 (-0.051, 0.113)	0.043 (-0.045, 0.131)	-0.065 (-0.126, -0.004)*	-0.056 (-0.119, 0.008)	-0.055 (-0.119, 0.009)	-0.048 (-0.115, 0.020)
	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase	Ratio (95% CI) per one SD increase
Firmicutes:Bacteroidetes †	1.03 (0.848, 1.26)	1.14 (0.947, 1.37)	1.14 (0.945, 1.37)	1.05 (0.864, 1.28)	0.872 (0.728, 1.05)	0.838 (0.694, 1.01)	0.838 (0.693, 1.01)	0.828 (0.677, 1.01)

Model 1: Unadjusted. Model 2: Adjusted for age, deprivation index, body fat % group. Model 3: Model 2 + also adjusted for sleep quality (PSQI global score), dietary fibre intake (g/day). Model 4: Model 3 + also adjusted for moderate to vigorous physical activity (min/day)

*P value <0.05, **P value ≤0.01, ***P value ≤0.001. † data has been log transformed (*ln*). Pacific, participants who identify as Pacific. NZE, participants who identify as New Zealand European.

Regression coefficients (β) represents one standard deviation higher sedentary behaviour (1.45 hours/day). Regression coefficients (β) obtained from log-transformed data represent relative differences and were expressed as a ratio (by using e^{β}).

The odds of being characterised by enterotype 1, 2 or 3 was no different between women who met the PA guidelines and those who did not meet the PA guidelines and those spending increased time in MVPA (Table 7.7). After controlling for all potential confounders, for every 1 SD increase in total PA (197 cpm/day) the odds among NZE women of being characterised by enterotype 1 were 1.623 times greater than being characterised by enterotypes 2 and 3, and the odds of being characterised by enterotype 3 were 41.6% lower compared to enterotype 1 and 2. After controlling for confounders (including time spent in MVPA), for every 1 SD increase in SB (1.45 hours/day) the odds of NZE women characterised by enterotype 1 was 39.4% lower compared with enterotype 2 and 3, and the odds of being characterised by enterotype 3 were 1.829 higher compared with enterotype 1 and 2.

Table 7.7 Odds of being characterised by enterotypes 1, 2 and 3 with increased time spent in physical activity, sedentary behaviour and meeting the physical activity guidelines

	Pacific					**NZE**				
Enterotype	Model 1 119	Model 2 117	Model 3 115	Model 4 115	Model 5 115	Model 1 159	Model 2 158	Model 3 157	Model 4 157	Model 5 157
Total PA	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Enterotype 1	1.17 (0.770, 1.78)	1.23 (0.795, 1.90)	1.20 (0.771, 1.88)			1.59 (1.13, 2.24)**	1.62 (1.13, 2.32)**	1.62 (1.13, 2.34)**		
Enterotype 2	0.802 (0.526, 1.22)	0.751 (0.479, 1.18)	0.766 (0.483, 1.22)			0.885 (0.460, 1.70)	0.986 (0.495, 1.96)	1.04 (0.536, 2.00)		
Enterotype 3	1.25 (0.579, 2.68)	1.29 (0.567, 2.91)	1.26 (0.555, 2.85)			0.635 (0.447, 0.903)*	0.595 (0.409, 0.866)**	0.584 (0.397, 0.859)**		
MVPA										
Enterotype 1	1.38 (0.661, 2.89)	1.31 (0.816, 2.09)	1.30 (0.802, 2.10)	1.21 (0.454, 3.24)	1.26 (0.517, 3.09)	1.19 (0.863, 1.63)	1.18 (0.850, 1.65)	1.16 (0.819, 1.63)	0.987 (0.680, 1.43)	1.07 (0.745, 1.53)
Enterotype 2	0.659 (0.413, 1.05)	0.689 (0.415, 1.14)	0.694 (0.415, 1.16)	0.767 (0.444, 1.33)	0.753 (0.444, 1.28)	0.827 (0.419, 1.63)	0.857 (0.425, 1.73)	0.935 (0.455, 1.92)	0.875 (0.409, 1.87)	0.941 (0.452, 1.96)
Enterotype 3	1.38 (0.661, 2.89)	1.22 (0.556, 2.68)	1.28 (0.544, 3.00)	1.21 (0.454, 3.24)	1.26 (0.517, 3.09)	0.874 (0.630, 1.21)	0.866 (0.615, 1.22)	0.870 (0.609, 1.24)	1.06 (0.715, 1.56)	0.948 (0.655, 1.37)
Sedentary behaviour										
Enterotype 1	0.853 (0.599, 1.22)	0.795 (0.546, 1.16)	0.794 (0.542, 1.17)	0.837 (0.554, 1.26)		0.631 (0.448, 0.887)**	0.601 (0.418, 0.863)**	0.609 (0.424, 0.874)**	0.606 (0.414, 0.888)*	
Enterotype 2	1.195 (0.837, 1.707)	1.331 (0.904, 1.960)	1.348 (0.904, 2.010)	1.257 (0.822, 1.922)		1.017 (0.535, 1.933)	0.868 (0.440, 1.712)	0.857 (0.444, 1.651)	0.826 (0.419, 1.628)	
Enterotype 3	0.936 (0.487, 1.801)	0.830 (0.402, 1.712)	0.841 (0.407, 1.739)	0.909 (0.395, 2.093)		1.623 (1.140, 2.310)**	1.808 (1.232, 2.654)**	1.797 (1.222, 2.643)**	1.829 (1.217, 2.746)**	
Meeting PA guidelines										
Enterotype 1	0.585 (0.281, 1.22)	0.570 (0.266, 1.22)	0.613 (0.281, 1.34)			0.935 (0.460, 1.90)	0.900 (0.436, 1.86)	0.974 (0.463, 2.05)		
Enterotype 2	2.010 (0.958, 4.22)	2.104 (0.950, 4.66)	1.92 (0.849, 4.35)			0.681 (0.139, 3.35)	0.579 (0.113, 2.96)	0.489 (0.091, 2.61)		
Enterotype 3	0.575 (0.146, 2.23)	0.597 (0.143, 2.49)	0.593 (0.139, 2.53)			1.17 (0.569, 2.41)	1.29 (0.614, 2.72)	1.24 (0.580, 2.66)		

Model 1: Unadjusted. Model 2: Adjusted for age, deprivation index, body fat % group. Model 3: Model 2 + also adjusted for sleep quality (PSQI global score), dietary fibre intake (g/day). Model 4: Model 3 + also adjusted for sedentary behaviour (for assessing MVPA) or MVPA (for assessing sedentary behaviour). Model 5: Model 3 + also adjusted for light physical activity (min/day)

*P value <0.05, **P value ≤0.01, ***P value ≤0.001. [†] Data has been log transformed (*ln*). Pacific, participants who identify as Pacific. NZE, participants who identify as New Zealand European. PA, physical activity. MVPA, moderate to vigorous physical activity. OR (e^{β}), odds ratio; 95% CI, 95% confidence interval.

7.5 Discussion

We aimed to test whether PA, including meeting the WHO PA guidelines, demonstrated an association with the gut microbiota α-diversity and taxonomic composition at the family level, of healthy Pacific and NZE women. To our knowledge, only one other study has investigated differences in taxonomic composition of the gut microbiota in relation to meeting PA guidelines (Bressa et al., 2017). Bressa et. al (2017). investigated the gut microbiota of sedentary and physically active (WHO guidelines) premenopausal Caucasian women (Bressa et al., 2017). Whilst we found no significant differences in gut microbiota family relative abundance between those who met the PA guidelines and those who did not, Bressa et. al. (2017) identified significant differences in the relative abundances of *Barnesiellaceae* (p=0.001) and *Odoribacteraceae* (p=0.009), with a higher presence of these bacterial families observed in sedentary women. However, the aforementioned families' relative abundances were very low, 0.86% and 0.66% respectively within their sedentary population, and it is unknown whether this would elicit physiological response. The relative abundance of *Barnesiellaceae* and *Odoribacteraceae* in our study were also very low (<1%) and did not feature in the most abundant 10 bacteria families thus were not included in our analysis. Bressa et. al. (2017) also found participants in the sedentary group consumed significantly less fibre and more processed meat when compared to the active group. Therefore, differences observed in gut microbiota of sedentary and physically active participants, may in fact be due to differences in dietary intake, in particular fibre, rather than PA. We have previously shown that within this population, meeting PA guidelines was not associated with total energy, total carbohydrate (sugar or starch), or fibre intake (Chapter 6). This gives us confidence that the consumption of these nutrients are likely not confounding our results. However it is important to recognise fibre is a broad category and it is possible one of its subcategories (e.g. pectin, psyllium, beta-glucans, lignin and cellulose) could be confounding our results.

We also investigated whether total PA, MVPA and SB were associated with the human gut microbiota. It was interesting to find among NZE women, every one SD increase in total PA (197 cpm/day) was associated with 36.3% higher relative abundance of *Erysipelotrichaceae* (p=0.031) and 37.9% lower relative abundance of *Verrucomicrobiaceae* (p=0.029). Significantly higher *Erysipelotrichaceae* abundances have previously been reported in elite athletes when compared to controls (Clarke et al., 2014b). Estaki et al. (2016) investigated the gut microbiota of individuals with varying levels of cardiorespiratory fitness measured by peak

oxygen uptake. Using dietary recall method, participant's diets were matched and it was demonstrated that cardiorespiratory fitness was positively correlated with faecal counts for a number of bacteria from the dominant phylum *Firmicutes* (specifically; family *Lachnospiraceae* and family *Erysipelotrichaceae*) and a higher production of the SCFA, butyrate. Whilst Estaki et al's. (2016) finding supports the thought that increased physical fitness may support overall gut homeostasis and health through changes in the gut microbiota we cannot draw such conclusions from the present study. Some bacteria in the *Erysipelotrichaceae* family are known to be capable of butyrate production, however the present study as well as the study by Clarke et al. (2014a) did not collect measurements of faecal SCFA. Therefore, we do not know if the increased relative abundance of *Erysipelotrichaceae* is translated into significant changes in the amount of SCFA butyrate produced (bacterial fermentation). Thus, whilst it is interesting to find these significant positive associations between increased total PA and reduced SB and the relative abundance of the bacterial family *Erysipelotrichaceae* in a non-athletic population that participated in low levels of PA, we cannot speculate on the biological meaning of these results. Furthermore, the relative abundance of *Erysipelotrichaceae* in the current study's population was low.

After controlling for potential confounders, this study found no significant association between increased total PA, MVPA or SB and α-diversity (Predicted species abundance, Pielou's Evenness and Shannon index). However interestingly, for NZE women who did not meet the PA guidelines, the Pielou's Evenness (-0.025, p=0.013) and Shannon Index (-0.155, p=0.031) were significantly lower compared to NZE women who did meet the PA guidelines. Investigating community-dwelling older men, objectively measured PA (step count) was not associated with α-diversity of the gut microbiota (Shannon index and the Inverse Simpson index) (Langsetmo et al., 2019). However, contrary to these findings, Barton et al. (Barton et al., 2018) reported gut bacterial α-diversity (Shannon Index) to be higher in professional male rugby players compared with healthy controls. Also, it has been demonstrated that cardiorespiratory fitness is positively correlated with increased gut microbial α-diversity (species richness, chao1, Shannon, Simpson, and Faith's phylogenetic diversity) (Estaki et al., 2016). Combined, these results suggest PA may contribute to increased α-diversity of the human gut microbiota, even at the WHO's minimum recommended level of PA. Considering greater gut microbiota diversity has been associated with improved health (Claesson et al., 2012) and likewise, lower diversity has been associated with poorer health outcomes such as obesity, T2D, irritable bowel disease (Huttenhower et al., 2014) and some cancers (Ahn et al.,

2013), it would be valuable for future research to determine the sufficient amount and intensity of PA to be associated with increased gut microbiota diversity in Pacific women.

A higher *Firmicutes:Bacteroidetes* ratio has been associated with obesity, thus it is of interest to investigate whether PA is associated with the ratio of these bacteria in the human gut (Ley et al., 2005). In this study, among Pacific women, not meeting the PA guidelines was associated with a 54.2% (p=0.026) higher *Firmicutes:Bacteroidetes* ratio compared to Pacific women who did meet the PA guidelines and increasing time spent in MVPA was significantly associated with a lower (p=0.034) *Firmicutes:Bacteroidetes* ratio, among Pacific women. Whereas among NZE women increased total PA was significantly associated with higher *Firmicutes:Bacteroidetes* ratio (p=0.031). It was interesting to find these associations were in opposite directions for Pacific and NZE women. Indeed previous research in humans and animals investigating this association have found also varying results; a reduction in *Firmicute*s and/or an increase in *Bacteroidetes* as a result of PA has been reported (Queipo-Ortuño et al., 2013, Denou et al., 2016), whilst others showed the opposite effect (Choi et al., 2013, Lambert et al., 2015, Clarke et al., 2014a), or no effect (Bressa et al., 2017, Munukka et al., 2018). Among professional athletes, lower presence of the *Bacteroidetes* population has been detected with increased PA (Clarke et al., 2014a). Investigating healthy young adults, Durk et. al. (2019), reported a significant positive association between *Firmicutes:Bacteroidetes* ratio and cardiorespiratory fitness (r = 0.48, p<0.003), but no other fitness, dietary intake, or anthropometric variables (p>0.004; significance was set at p<0.004). However, no difference in *Firmicutes:Bacteroidetes ratio* was found between active and non-active individuals when investigating a non-athletic population (Bressa et al., 2017). These results may indicate this associations between PA and relative abundance of gut bacteria are much more specific than at the broad phylum level and investigations at the genera or species level may draw more consistent and clear results.

In this study Pacific women with a high BF% had a significantly higher *Firmicutes:Bacteroidetes* ratio compared to all other groups. There may be certain bacteria in the *Firmicutes* phyla that respond more to PA than bacteria within the *Bacteroidetes* phyla (Dorelli et al., 2021), therefore it would be interesting to investigate bacteria within these phyla at a more detailed level such as at the species level. There may be certain species of bacteria within these phyla that are associated with are more dominant in Pacific or NZE women. Therefore their capacity to increase or decrease with PA would be greater in one ethnic group when compared to the other. Importantly, mechanisms by which *Firmicutes* and *Bacteroidete*s,

can affect human phenotypes remain to be fully elucidated (Johnson et al., 2017), therefore this finding should be interpreted with caution. However this finding should be further investigated in future research as may suggest PA association with gut microbiota composition (in particular bacteria within the *Firmicutes* and *Bacteroidetes* phyla) differs between ethnic groups.

Lastly, a decade ago (2011) researchers classified the human gut microbiota into three taxonomic clusters or 'enterotypes'(Arumugam et al., 2011). These enterotypes were based on the abundance of key bacterial genera, namely the *Prevotella*, *Bacteroides* and *Ruminococcus* enterotypes. It was suggested that bacterial members of each enterotype co-exist for functionality (Arumugam et al., 2011). These enterotypes have since been associated with habitual dietary intake, for example a carbohydrate-rich diet, rather than meat and dairy, has been associated with the *Prevotella* enterotype (Wu et al., 2011). However, it is unknown whether PA is also associated with enterotype within a population. In this study we found no difference in meeting the PA guidelines between women who were characterised by the three, distinct enterotypes previously identified in the PROMISE study population. We did however find among NZE women, increased time spent in total PA (cpm) was associated with greater odds of being characterised by enterotype 1 compared to enterotype 2 and 3 and significantly lower odds of being characterised by enterotype 3 compared to enterotype 1 and 2. Whilst increased time spent in SB was associated with the opposite (significantly lower odds of being characterised by enterotype 1 compared to enterotype 2 and 3 and a significantly greater odds of being characterised by enterotype 3 compared to enterotype 1 and 2). This is an interesting finding as enterotype 1 is characterised by butyrate-producing bacterial species (*Eubacterium rectale and Faecalibacterium prausnitzii*). Thus this research adds to the evidence suggesting that PA is positively associated with relative abundance of bacteria that promote the production of gut microbiota metabolites, like SCFAs (Clark and Mach, 2017). Importantly these associations were found in a non-athletic population who participate in low levels of PA, reiterating the importance of encouraging all physical movement and discouraging SB. Although enterotype 1 was found in both Pacific and NZE women, this association was not observed among Pacific women in this study. Total PA did differ between Pacific and NZE women, with Pacific women with a low-BF% participating in significantly less total PA compared to NZE women. Therefore this may have contributed to the lack of significant associations between PA and SB variables and presence/absence of enterotypes 1-3 among Pacific women. It would also be interesting for future research to investigate other possible

reasons why, this association was not found among Pacific women, such as potential differences in type of PA that Pacific and NZE women are participating in.

In comparison, Bressa et. al. also classified their population by enterotype, finding the majority of their population was in the *Bacteroides* enterotype but did not mention the ratio of physically active to non-active participants within this group (Bressa et al., 2017). The remaining participants belonged to the *Prevotella* enterotype, with two thirds (66%) of this population in the active group who met the PA guidelines. None of their participants were classified in the *Ruminococcus* enterotype.

Comparing evidence across this research field is difficult. The heterogeneity of methodological approaches with regards to measurement of PA is a challenge and may also be a factor contributing to lack of consistency in results. A strength to our study was the use of a valid, objective measure of PA and SB (accelerometer) to clearly define the participant's PA and SB profiles. Most studies use self-reported questionnaires to determine PA (Petersen et al., 2017, Barton et al., 2018, Mörkl et al., 2017, Clarke et al., 2014b), only some of these being validated (Clarke et al., 2014b, Mörkl et al., 2017, Barton et al., 2018). A few others have used objective measures of PA such as accelerometers (Langsetmo et al., 2019) (Bressa et al., 2017). None-the-less, there is some consistency in results between studies. Significant findings in this research area appear to be less robust in studies performed in the general population compared to trained individuals taking part in prolonged, intense exercise. This may be because when only looking in trained individuals there is likely less variability in PA, as compared to the general population, therefore when investigating the general population there is less power to detect a difference. Adding to this, athletes and individuals who perform at high levels of PA often have a different diet when compared to the general public (Clarke et al., 2014a), which can favour specific microbial populations (Clark and Mach, 2016, Shahar et al., 2020, Mitchell et al., 2019). Therefore, it is difficult to disentangle the effects of PA from the effects of diet on the microbiota, emphasising the importance of carefully controlling for dietary intake in statistical analyses. The use of a prospective food record was a strength of our study, which allowed collecting detailed information on dietary intake, whilst participants did not have to rely on memory. This allowed us to confidently control for dietary intake in our statistical analysis.

Cross-sectional research such as the present study is purely descriptive and only represents a snapshot in time. Longitudinal studies will allow lifestyle factors such as diet and sleep to be controlled for in more comprehensively to help determine whether PA independently alters the

gut microbiota in humans. Importantly more mechanistic studies, such as animal models, are needed to clearly determine the mechanism/s (if any) that are responsible for the adaptation of the gut microbiota to PA. Further, to also investigate whether PA induced changes in the gut environment are potentially disease modifying. This will assist in designing future research that is more targeted to specific bacteria as well as types of PA such as resistance vs endurance. SCFA's, including butyrate, play a significant role in gut and general health, including acting as an energy source for microbes, modulating inflammation, and gut motility (Liu et al., 2022). Considering this, more knowledge is required about the possible mechanisms in which PA may increase SCFA's in the gut. Also, what relative abundance of SCFA's such as butyrate producing bacteria from the *Erysipelotrichaceae* family is required to reach a level of butyrate in the gut that would translate to improved human metabolic health? Having this knowledge would allow future studies (including cross-sectional research) investigating PA and the gut microbiota composition to be more targeted and interpreted more clearly.

Furthermore reproducibility of results in this area of research is low. Human studies supporting our finding of higher relative abundance of the *Erysipelotrichaceae* family with increased PA or reduced SB are minimal and the confounding factor of diet makes these results questionable (Clarke et al., 2014a). Furthermore, in the present study, associations between PA and the *Firmicutes:Bacteroidetes* ratio within our own population differed between Pacific and NZE women. This also emphasises that more needs to be known about the mechanisms driving associations between PA, SB and the gut microbiota to enable past and future research findings to be interpreted more clearly.

The intimate relationship between PA and diet means the known confounding influence of dietary intake must be carefully considered alongside PA assessment (Turnbaugh et al., 2006, Yang et al., 2020). Although many studies gather dietary intake information, they fail to control for its confounding effect (Barton et al., 2018, Durk et al., 2019, Mörkl et al., 2017, Estaki et al., 2016, Clarke et al., 2014b). Further, none of the aforementioned studies controlled for the possible confounder of sleep behaviour. Considering that the evidence highlights the impact of sleep behaviour on the gut microbiota, it is an important factor that should be controlled for in future research (Thaiss et al., 2014). Research also needs to continue to assist in identifying the specific PA patterns, including intensity and duration, unique to certain geographical locations and cultures that play a role in shaping the microbiota. In turn, this will allow a more comprehensive understanding of the interaction between PA and the gut microbiota, to improve the health status of all humans.

7.6 Conclusion

The results presented in this research suggest increased time spent in total PA and reduced time spent sedentary was positively associated with relative abundance of bacteria capable of producing SCFA's, especially among NZE, when compared to Pacific women. Associations between PA and gut microbiota composition (in particular bacteria within the *Firmicutes* and *Bacteroidetes* phyla) may differ between ethnic groups. More needs to be known about the mechanisms driving associations between PA, SB and the gut microbiota to enable past and future research to be interpreted more clearly.

7.7 References

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Chapter 8: Discussion - summary of research questions and findings

This thesis aimed to improve understanding of the relationships between physical activity (PA) and sedentary behaviour (SB) on body composition, markers of metabolic health and the gut microbiota, in a population of Pacific and New Zealand European (NZE) women, aged 18-45 years, living in New Zealand (NZ). This chapter provides an overview of the collective findings of this thesis. Implications and limitations of these findings, as well as directions for future research are also discussed.

8.1 Summary of the study

The research in this PhD was part of the larger cross-sectional "PROMISE" study. Data was collected over a 14-month (July 2016 – September 2017) period, covering all four seasons. A total of 351 women took part in the PROMISE study (Kindleysides et al., 2019). Of these women, 278 provided valid accelerometer data, and were eligible to take part in this research: 119 Pacific (43%) and 159 NZE (57%) women were included in the analysis.

Due to time, cost and participant burden, self-reported measures (e.g. questionnaires) tend to be selected to estimate PA. Compared to objective measures (e.g. accelerometers), self-reported measures of PA are less accurate in women (Ferrari et al., 2007). Self-reported measures of PA are also less accurate in providing accurate measures of light PA, due to it being so dominant in everyday life, such as household activities (e.g. vacuuming, making a bed, etc.) (Skender et al., 2016). Therefore, in the present study PA data was collected objectively using accelerometers. Although no objective measure of dietary intake is currently available, multiple days of prospective dietary assessment can reduce the likelihood of misreporting, compared to memory-based, retrospective assessment tools (Willett, 2012, Gibson, 2005). The current study used a 5-day non-consecutive, estimated food record to obtain an accurate record of actual dietary intake over the data collection period that covered a range of week and weekend days. Alongside this, detailed assessment of body composition (including BF% measured by DXA scan), a comprehensive range of blood chemistry and other clinically relevant markers of metabolic health (e.g. blood pressure), were assessed. Lastly, faecal samples were collected and metagenomic shotgun sequencing was conducted to explore the composition of participants' gut microbiota. These data were used to describe PA and SB patterns of our population and to investigate the associations of PA, and SB on their body composition, metabolic health and gut microbiota composition.

It is well established that increased BF% is associated with a poorer metabolic health profile (World Health Organisation, 2021). This research corroborates this, also finding that women with a high-BF% were at higher risk of poorer metabolic health when compared to women with a low-BF% (Pacific; triglycerides, HbA1c, plasma insulin, leptin and NZE; triglycerides, HbA1c, plasma insulin, plasma glucose, LDL, leptin all significantly higher ($p<0.05$)). Measures of adiposity (waist circumference, BMI) have also been associated with lower levels of PA and higher SB (Henson et al., 2013, Healy et al., 2008, Rosique-Esteban et al., 2017, Gibbs et al., 2017). In this study there was no difference in total PA or light PA between women in the high- or low-BF% group, however women with a low-BF% spent significantly more time in the intense MVPA (Pacific high- (19 min/day) and low-BF% (26 min/day) ($p<0.05$) and NZE high- (31 min/day) and low-BF% (39 min/day, data from Chapter 4) ($p<0.05$). High-BF% NZE but not Pacific women had a higher weighted median sedentary bout length (20.07 min) compared to their low-BF% counterparts (NZE low-BF% (17.81 min)) ($p<0.05$). Whilst there was no difference in time spent sedentary between NZE BF% groups, an interesting finding from this research was that Pacific women with a low-BF% spent significantly more times sedentary compared to Pacific women with a high-BF% (low- 10.37 and high-BF% 9.93 h/day). It would be interesting for future research to investigate this further, including what types of SB this group of women are participating in, such as sitting on the couch watching TV or more active SB, such as typing on a computer, and if this differs to other groups of women, in particular Pacific women with a high-BF%.

Furthermore, minority ethnic groups have been found to have lower levels of PA and higher levels of SB (Langøien et al., 2017). In this research participation in total PA and light PA did not differ between Pacific and NZE women, however, Pacific women did spend significantly less time in MVPA (Pacific high- 19.1 and low-BF% 26.3 min/day) compared to NZE women (NZE high BF% 30.5 and low-BF% 39.1 min/day) ($p\leq0.05$) and low-BF% Pacific women spent more time sedentary than all other women ($p<0.05$) (Pacific high-BF% 9.93 and low BF% 10.37 and high-BF% 9.96 and NZE low-BF% 9.69 min/day).

Differences between ethnic groups are also seen in the prevalence and risk of chronic conditions (Hales CM, 2020, Quiñones et al., 2019). Specifically, in NZ, compared to NZEs, Pacific people have an increased risk of T2D (Sundborn et al., 2008); the prevalence of diabetes is 2.0 times higher among Pacific compared to NZE (Ministry of Health New Zealand, 2021).

Whilst there was no difference between Pacific and NZE women in total or regional BF% in the high-BF% group; when comparing the low-BF% groups, NZE women had lower total, android, gynoid, visceral and trunk fat percentages ($p<0.05$). This difference in regional BF%, may contribute towards Pacific women's increased susceptibility to metabolic diseases (Swindell et al., 2018, Healy et al., 2008, Henson et al., 2013), in particular central adiposity such as visceral fat, due to its association with increased risk of non-communicable diseases and inflammation (Zhang et al., 2015, Fontana et al., 2007, Kang et al., 2015, Fox et al., 2007, Stępień et al., 2014). Additionally, despite no significant difference in BF% among Pacific and NZE women in the high-BF% group, significant differences in some metabolic markers were seen between these groups of women as well as women in the low-BF% groups. In this research all participants had normal HbA1c levels below 41 mmol/mol (Labtests, 2021), and had not been diagnosed with diabetes. However Pacific women did appear to be at increased risk of developing T2D as their fasting plasma insulin concentrations were almost two-fold higher than that of their NZE counterparts ($p \leq 0.05$), and they also had significantly higher HbA1c levels ($p \leq 0.05$). Additionally, plasma glucose concentrations were higher among Pacific women with a high-BF% than for NZE with a high-BF% ($p<0.05$) and among women with a high-BF%, NZE women had increased CRP and LDL when compared to Pacific women ($p<0.05$).

Also consistent with previous research, Pacific women in this study spent less time in PA when compared to NZE women (O'Brien, 2018, O'Brien et al., 2019) and Pacific women with a low-BF% spent more time in SB compared to NZE with a low-BF%. Higher levels of objectively measured PA and lower levels of SB were associated with reduced metabolic risk markers in both Pacific and NZE women. However, the aforementioned baseline differences in metabolic markers between Pacific and NZE women appear to influence these associations, this is discussed in the following paragraphs.

8.2 Discussion of the main results by research objectives

The first objective of this PhD thesis was to determine if objectively measured PA was associated with different metabolic health risk and body fat profiles and whether these associations differ between Pacific and NZE women.

H^1 - Increased time spent in physical activity (MVPA, light and total PA) would be associated with lower metabolic risk and lower BF%, and that these associations would not differ between Pacific and NZE women.

In this study, increased MVPA was associated with lower total body, trunk and gynoid fat% in both Pacific and NZE women (**H[1] accepted; for body composition**). Interestingly, higher total PA and MVPA were inversely associated with visceral and android fat % amongst NZE, but not Pacific women. This finding is important, as Pacific women had significantly higher visceral adiposity when compared to NZE women, possibly contributing to increased risk of non-communicable diseases (Zhang et al., 2015, Fontana et al., 2007, Kang et al., 2015, Fox et al., 2007, Stępień et al., 2014). As previously mentioned, time spent in MVPA did differ between Pacific and NZE women and Pacific women in the low-BF% group participated in significantly less total PA when compared to NZE women in the low-BF% group ($p \leq 0.05$). Therefore, it is possible Pacific women may require higher levels of MVPA and/or total PA than observed in this study to experience a similar associated lower total and regional BF% as observed in NZE women. This warrants further investigation. Additionally, this research did not investigate the type of PA (e.g. resistance weight training, walking or running) which may play a role in the association between PA and body composition. Previous research has shown that Pacific women prefer to take part in different types of PA compared to NZE; whilst both ethic groups reported high participation in walking, Pacific women also reported music and dance among their top four activities whereas NZE women reported taking part in "gym-type activities" (O'Brien et al., 2019).

Increases in both total PA and MVPA were associated with lower common clinical markers of metabolic disease risk, specifically lower fasting plasma insulin, C-reactive protein (CRP) and heart rate. For both Pacific and NZE women, there was an associated 8% lower fasting plasma insulin concentration for every 10-minute increase in MVPA. Every 100 cpm increase in total PA was also associated with a 9% lower fasting plasma insulin among Pacific but not NZE women, suggesting the impact of increased total PA on fasting insulin may be greater in Pacific women who are a high metabolic disease risk population, therefore this could potentially provide an effective avenue for intervention (**H[1] accepted; for biomarkers of metabolic health**).

The second research objective was to 1) describe patterns of sedentary behaviour among Pacific and NZE women. Further to determine if 2) objectively measured SB was associated with different metabolic risk and body composition; and 3) whether these associations differ between Pacific and NZE women.

H^2: Increased time spent in SB, and prolonged bouts of SB would be associated with increased BF% and higher metabolic risk, and that these associations would not differ between Pacific and NZE women.

As a population, women in this study had high levels of SB (Pacific low-BF% 10.37 h/day and high-BF% 9.93 h/day and NZE low-BF% 9.69 h/day and high-BF% 9.96 h/day). Our findings indicate breaking-up prolonged SB may assist in achieving healthier BF%. Among NZE women, decreased time spent sedentary was associated with lower gynoid fat ($p<0.05$), and lower weighted median sedentary bout length was associated with lower BF% (gynoid fat, total body, trunk, android and visceral fat ($p<0.05$)). No associations between SB and body composition were found among Pacific women. (**H^2 accepted for body composition among NZE women; H^2 rejected for body composition among Pacific women**). With regard to biomarker outcomes, among NZE women every one-hour increase in sedentary time was associated with a 14% higher CRP ($p<0.05$). These results suggest inflammation may be an additional pathway through which increased sedentary time may impact on metabolic risk, especially among NZE, when compared to Pacific women (**H^2 accepted for biomarkers of metabolic health among NZE women; H^2 rejected for biomarkers of metabolic health among Pacific women**).

Based on our findings these high levels of SB may be a contributor to increased BF% and/or reduced metabolic health, depending on ethnicity. Our research adds to the paucity of evidence on the associations between objectively measured SB (duration and patterns) and body composition and metabolic biomarkers in healthy adult women. This research also builds on the limited research on SB in ethnic minorities (Schmid et al., 2018, Katzmarzyk et al., 2019). The finding that Pacific women with a low-BF% (a minority ethnic group within NZ) participated in higher levels of SB when compared to NZE women, is in agreement with previous research that found minority groups, including indigenous populations in Europe and immigrants from low and middle income countries, had higher levels of SB (Langøien et al., 2017). This is an important finding because whilst qualitative SB guidelines exist, research in this area needs to expand to include wider populations in society before public health officials can confidently publish quantitative SB guidelines. These findings suggest it may be of benefit for these quantitative SB guidelines to differ depending on ethnicity.

The third research objective was to explore associations between nutrient intakes and meeting the PA guidelines and whether this differs between ethnicities.

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H[3] - *We hypothesise that meeting the PA guidelines will be associated with a healthier diet from a nutrient perspective (higher percentage of participant meeting AMDR's and higher micronutrient intakes) compared to not meeting the PA guidelines, and that this would not differ between Pacific and NZE women.*

Dietary intake and PA are both known to be associated with the development of obesity and non-communicable diseases and this study added to the limited evidence on the association between these lifestyle factors across different body composition profiles. All the women that were in the lowest quartile of fibre intake, and particularly the NZE women, had a lower odds of meeting the PA guidelines (OR 0.72 (p=0.008) and OR 0.66 (p=0.021) respectively) compared to women in the top three quartiles of fibre intake. Additionally, all the women that were in the lowest quartile of polyunsaturated fat intake (when measured as a % of total energy intake), especially Pacific women had a lower odds of meeting the PA guidelines compared women in the top three quartiles (0.76, p=0.027 and OR 0.67, p=0.030 respectively). The remainder of the findings from Chapter 6 indicate that among Pacific and NZE women in this population, meeting the PA guidelines was not associated with diet composition from a nutrient perspective (**H[3] rejected**). Physical activity induces changes in hormones implicated in appetite regulation (including leptin, ghrelin, peptide YY, glucagon-like peptide-1 and insulin) (Schubert et al., 2014, Gondim et al., 2015). Therefore, being habitually physically active allows individuals to have a heightened sensitivity to their appetite regulation, allowing them to better match energy intake with energy expenditure (Hopkins and Blundell, 2016, Dorling et al., 2018, Blundell et al., 2003). Populations of athletes have been shown to have higher protein intakes compared to non-athlete controls (Clarke et al., 2014a) and higher fibre intakes have been found among women who meet the PA guidelines compared to women who do not (Bressa et al., 2017). Consumption of both protein and fibre appear to have positive effects on modulating satiety and energy metabolism (San-Cristobal et al., 2020). It may be that the level of PA needed to achieve the PA guidelines is too low to be associated with any difference in dietary intake within our population. Although results did not reveal any key findings at the nutrient level it, would be interesting to take this research a step further and investigate any associations between meeting the PA guidelines and dietary patterns. This would help to provide a deeper understanding of the relationship between food choice and PA.

The fourth and final research objective was to explore the possibility that PA and SB (MVPA, total PA, sedentary behaviour and/or meeting PA guidelines *versus* not meeting PA guidelines)

are associated with broad taxonomic composition (family level) and α-diversity of gut microbiota that could drive beneficial metabolic changes to human hosts.

H^4: We hypothesise that increased time spent in PA, reduced time spent in SB and meeting the PA guidelines will be associated with increased α-diversity and relative abundance of health promoting bacteria (family level) in the human gut.

H^4 - *Increased time spent in PA, reduced time spent in SB and meeting the PA guidelines will be associated with increased α-diversity and higher relative abundance of health promoting bacteria (family level) in the human gut.*

This study did not find an association between increased total PA, MVPA, SB or meeting/not meeting the PA guidelines and α-diversity among Pacific women (Predicted species abundance, Pielou's Evenness and Shannon index) (**H^4 rejected among Pacific women; for α-diversity**). However, among NZE women, those who did not meet the PA guidelines Pielou's Evenness (-0.025, p=0.013) and Shannon Index (-0.155, p=0.031) were significantly lower compared to women who did meet the PA guidelines (**H^4 accepted among NZE women; for α-diversity**). Previous research has demonstrated that PA is associated with increased gut microbiota α-diversity among athletes. For example, when division one collegiate swimmers were assessed during peak training through their in-season taper, where they had a reduction in swim distance per week (32.6±4.8 km/week to 11.3±8.1 km/week) (Hampton-Marcell et al., 2020), a significant decrease in microbial α-diversity was observed. Additionally bacterial α-diversity (Shannon Index) has been shown to be higher in professional male rugby players compared with healthy controls (Barton et al., 2018). However considering greater gut microbiota diversity has been associated with improved health, it was encouraging to see this association at the minimal level of PA recommended by the World Health Organisation (WHO) and in a non-athletic population (Claesson et al., 2012) and lower diversity has been associated with obesity (Ley et al., 2006).

Another notable finding was that among NZE women, every one SD increase in total PA (197 cpm/day) was associated with 36.3% higher relative abundance of butyrate producing bacteria *Erysipelotrichaceae* (p=0.031) and 37.9% lower relative abundance of *Verrucomicrobiaceae* (p=0.029). Furthermore, every one SD increase in SB (1.45 hours/day) was also associated with a 28% lower relative abundance of *Erysipelotrichaceae* (p=0.030) (**H^4 accepted among NZE women; for relative abundance of health promoting bacteria (family level)**). Again, it is positive to see these associations in a non-athletic population who participated in low levels

of PA. However, neither PA nor SB were associated with the human gut microbiota relative abundance of bacteria at the family level among Pacific women (**H[4] rejected among Pacific women; for relative abundance of health promoting bacteria (family level**).

8.3 General discussion of main results

A notable finding was the significant inverse association between increasing total PA and lower fasting plasma insulin concentrations among Pacific women but not NZE women. This finding suggests that increased total PA may have a greater impact on Pacific women's fasting insulin concentrations, which were higher compared with NZE women. Pacific women in this study spent less time in PA when compared with NZE women, which aligns with previous research (O'Brien et al., 2019). This difference in time spent in PA may, at least in part, explain the stronger inverse association between MVPA, total PA and adiposity versus the majority of markers of metabolic health that was observed in NZE women. However, it also emphasises the importance of the association between total PA and insulin found among Pacific women, considering Pacific women's total PA was significantly lower than their NZE counterparts, and total PA was not associated with body composition in this group.

Considering CRP is an inflammatory marker associated with increased risk of poor metabolic health (Kaptoge et al., 2010) our finding that Pacific women had significantly lower CRP levels compared to NZE women is important and warrants further investigation. Furthermore, despite having a similar total BF% to NZE women, and spending similar (high-BF% group) or more (low-BF% group) time in SB, but less time in MVPA, it was also interesting to find no association between Pacific women's CRP and total PA, or SB, whereas significant associations among NZE women were observed (where SB was associated with higher and PA lower CRP levels). The findings that increasing total PA and MVPA were inversely associated with visceral BF% amongst NZE women, but not in our Pacific population may help to explain this as CRP is positively associated with visceral BF% (Sanip et al., 2013). These findings suggest inflammation may be an additional pathway through which increased sedentary time may impact on cardiovascular risk in women, especially NZE, when compared to Pacific women. The fasting plasma insulin concentrations among Pacific women were twice as high as those of the NZE women. Also, NZE women's CRP levels were significantly higher than that of Pacific women. Therefore, it is clear that the capacity for change was greater among each ethnic group for the different biomarkers. It is also important to note that time spent sedentary and in light PA were highly correlated (VIF = 11.7, tolerance = 0.085) in this

research. It is therefore difficult to disentangle the association of CRP (or any other variable) with light PA (an aspect of total PA) and sedentary time. For example, the association of CRP and sedentary time among NZE women may in fact be an association between spending less time in light PA rather than higher SB or vice versa.

This research also highlighted areas of concern which were pertinent to both Pacific and NZE women. For example, on average a large percentage of Pacific women and women with a high-BF% were not meeting the PA guidelines of 150-300 minutes MVPA per week (World Health Organisation, 2020) (% not meeting guidelines: Pacific: 53% low-BF% and 61% high-BF%; NZE: 19% low-BF% and 35% high-BF%). Also, whilst both Pacific and NZE women's carbohydrate intakes were within or below the acceptable macronutrient range; it was interesting to find that Pacific women consumed more starch as a percentage of total energy intake than their NZE counterparts Pacific: 25.2% low-BF% and 25.4% high-BF%; NZE: 20.8% low-BF% and 21.7% high-BF%). Additionally, mean total sugar intakes were at least 16% of total energy for all four groups (Pacific low-BF% 17.8% and high-BF% 17.5% and NZE low-BF% 16.0% and high-BF% 17.2%). The WHO recommends intake of free sugars to be <10% of total energy intake, however, in this study only total sugar intake was assessed (i.e. there is a marked difference between the nutrient density of fruit that contains naturally occurring sugars and a muesli bar with added/free sugars), therefore it cannot be compared to this guideline. It does however emphasise the importance of future research investigating the main food sources of each nutrient. Additionally, fibre intake was below the recommended daily intake (of ≥25 g/day) for Pacific women in both the high and low BF% groups (19.8 g/day and 19.2 g/day respectively) and NZE women in the high BF% group (22.1 g/day). Only the NZE low-BF% group's mean fibre intake was above this recommendation (26.2 g/day). Previous research has also found that compared to NZE women, Pacific women had lower intakes of dietary fibre and higher intakes of starch, when expressed in relation to total energy intake (Metcalf et al., 2008). Low fibre and increased sugar intake are known contributors to T2D (Afshin et al., 2019), as are low levels of PA (World Health Organisation, 2017). Common clinical markers for T2D are fasting plasma glucose, fasting plasma insulin and HbA1c. In this study, mean plasma glucose among women with high-BF% was higher among Pacific when compared to NZE women. Also compared to NZE women with low-BF%, Pacific women and NZE women with high-BF% had significantly higher HbA1c and fasting plasma insulin concentrations which may in part be explained by their higher visceral adiposity. With the knowledge that high-BF%, particularly central adiposity (Guh et al., 2009) mediates metabolic

outcomes such as T2D, and Pacific women are a high metabolic disease risk population with 9.5% diagnosed with diabetes (Ministry of Health New Zealand, 2021), promotion of PA in these populations must remain a priority.

Differences in obesity and metabolic disease prevalence between NZE and Pacific people are evident among adults in NZ (Ministry of Health New Zealand, 2021). However, the underlying reasons for these differences are yet to be fully understood. This research also highlights the role deprivation may be playing in this inequality in obesity and metabolic disease prevalence. In this study, ethnicity and BF% are confounded by level of deprivation, which is one of the most potent indicators of overall health not only in NZ (Ministry of Health New Zealand, 2021) but also the rest of the world (World Health Organisation, 2021).

In this study the population with the lowest metabolic disease risk were NZE women in the low-BF% group. These women were also less deprived in comparison to all Pacific women and NZE women in the high-BF% group, and also engaged in more MVPA and consumed more dietary fibre compared to their high-BF% and Pacific counterparts.

Populations at high risk of poor metabolic health often live in communities deprived of health promoting resources. They therefore experience inequality in the availability of PA resources, such as safe side walks (Gordon-Larsen et al., 2006) and higher food insecurity (Eagle et al., 2012, Cummins and Macintyre, 2006). Research has shown that a large portion of the association between deprivation and obesity is mediated through health behaviours such as PA and diet (Shaikh et al., 2015, Pampel et al., 2010). It has been shown that lower income groups often select cheaper diets that tend to be more energy dense and lack fruit and vegetables (Darmon and Drewnowski, 2015). Ultra-processed foods comprise the largest proportion of packaged foods in NZ supermarkets and have a worse nutrient profile than less processed foods (Luiten et al., 2016). There is also a lack of significant price difference between ultra- and less processed foods, making them as accessible as each other (Luiten et al., 2016). In NZ, living in the most deprived areas decreases the likelihood of consuming the recommended daily servings of fruit and vegetables by 30%, when compared to individuals living in the least deprived areas. Reduced food security, including poorer access to fruit and vegetables (Eagle et al., 2012, Cummins and Macintyre, 2006), and easy access to convenient ultra-processed foods, could lead to a poorer overall diet quality. Further, PA has a direct cost in terms of both time and resources such as money and access to PA promoting environments (Sport New Zealand, 2019); for example, access to safe places to exercise, safe side walks, bike paths and parks (Friel et al., 2007). O'Brien et al. (2019) found Pacific women with children spent

statistically less time in recreational activities than Pacific women without children (*p*=0.026), this finding was not replicated among NZE women (O'Brien et al., 2019). Furthermore, Pacific mothers have cited, “lack of time due to family responsibilities”; “I would have to get someone to get my children”; and “I have too many household chores to do” to be of the highest influence in regard to barriers to PA (Schluter et al., 2011).

On an individual level deprivation is arguably not modifiable, however in high income countries like NZ, deprivation is modifiable at a government level. This research highlights the need for public health strategies addressing obesity, including PA programming and policy, to reach women from low socioeconomic backgrounds to ensure it does not contribute to widening socioeconomic gaps in obesity-related health.

This research further contributes new knowledge as it is one of the first studies to include ethnic minorities, and the first to include Pacific women when investigating associations between PA and the gut microbiota. Although an optimal *Firmicutes:Bacteroidetes* ratio for human health has not been defined (Magne et al., 2020), a higher ratio of *Firmicutes:Bacteroidetes,* has been associated with obesity, T2D and altered blood glucose (Magne et al., 2020, Letchumanan et al., 2022). However evidence associating the proportions of bacterial phyla *Firmicutes* and *Bacteroidetes* with obesity, “is not convincing” and is still a matter of debate (Magne et al., 2020). None-the-less because it *may* have implications for the pathogenesis of obesity and T2D a finding that warrants further investigation is that increased total PA was associated with higher *Firmicutes:Bacteroidetes* ratio (23.1% for Every 1 SD increase (*p*=0.031)) among NZE women, whereas among Pacific women, increased time spent in MVPA was associated with a lower *Firmicutes:Bacteroidetes* ratio (22.8% for every 1 SD increase (*p*=0.034)). Although total PA and MVPA are different measures of PA, it was interesting to find these associations differed in direction between Pacific and NZE women and suggests associations between PA and gut microbiota composition (in particular bacteria within the *Firmicutes* and *Bacteroidetes* phyla) may differ between ethnic groups. It would be interesting to investigate bacteria within these phyla at a more detailed level such as at the species level. There may be certain species of bacteria within these phyla that are associated with are more dominant in Pacific or NZE women. Therefore their capacity to increase or decrease with PA would be greater in one ethnic group when compared to the other. It would also be interesting to investigate this association at higher levels of PA (both total PA and MVPA) in both ethnic groups.

Previous work by the PROMISE study characterised the composition of the participant’s microbiota by the presence of three enterotypes; enterotype 1, included both Pacific and NZE

women, whereas enterotype 2 was predominately found in Pacific women, and enterotype 3 predominately in NZE women (Renall, 2020). This study found NZE women who participated in more total PA were more likely to be characterised by enterotype 1 (1.62 times for every 1 SD increase in total PA) and less likely to be characterised by enterotype 3 (41.6% for every 1 SD increase in total PA). Additionally, with increased time spent in SB, NZE women were less likely to be characterised by enterotype 1 (39.4% less likely for every 1 SD increase) and more likely to be characterised by enterotype 3 (1.83 times more likely for every 1 SD increase). Combined with the finding of increased total PA and decreased SB being associated with higher relative abundance of the butyrate-producing bacteria *Erysipelotrichaceae* this is an interesting finding. Enterotype 1 is characterised by butyrate-producing bacterial species (*Eubacterium rectale and Faecalibacterium prausnitzii*), therefore this research supports the notion that increased time spent in PA and reduced time spent in SB is associated with the relative abundance of health promoting bacteria (family level) in the human gut (Clark and Mach, 2017). Furthermore, considering participants in the PROMISE study participated in low levels of PA these are important findings. Most previous research suggesting that PA is positively associated with relative abundance of bacteria that promote the production of gut microbiota metabolites, like SCFAs has been in athletic populations rather than the general public (Clark and Mach, 2017). Therefore, this research supports the importance of encouraging all physical movement and discouraging SB. It is important to note that although enterotype 1 was found in both Pacific and NZE women, no significant association between PA or SB and presence/absence of enterotype 1 to 3 was observed among Pacific women. Pacific women with a low-BF% were participating in significantly less total PA compared to NZE women with a low-BF%, therefore it is possible the levels of PA performed in this population may have been too low to detect any statistically significant associations. Furthermore, our sample size may have been too small to observe an association between PA and gut microbiota composition among Pacific women. However our sample size was larger than many other studies investigating associations between PA and the gut microbiota (Clarke et al., 2014a, Bressa et al., 2017, Petersen et al., 2017, Barton et al., 2018, Langsetmo et al., 2019, Hampton-Marcell et al., 2020). Furthermore, investigating the gut microbiota composition at a deeper level (relative abundance at the genus or species level) may reveal more significant findings.

8.4 Concluding remarks

A major risk factor for obesity and a leading cause of death in the world, physical inactivity is a pandemic (Kohl 3rd et al., 2012) and SB is increasingly associated with reduced metabolic health. Increased PA and reduced SB could greatly contribute to the health of NZ women through lowering the risk of obesity and reduced metabolic health. This PhD research presents several key and novel findings (listed below), that focus on these important areas of public health concern, in moderate and high metabolic disease risk populations. The results presented in this thesis emphasise the importance of increased PA and reduced SB as an integral part of a healthy lifestyle. In particular for the management of public health problems that currently affect all of NZ's ethnic groups such as obesity and T2D.

Within our population, there was an inverse association between total PA and key markers of metabolic health (fasting plasma insulin concentrations, CRP and heart rate). This is a notable finding as although other research in European populations have also reported increased total PA to be significantly associated with improved metabolic health including fasting plasma insulin concentrations, HOMA_IR, and CRP (Swindell et al., 2018, Ekelund et al., 2007), it is promising to also see metabolic benefits of total PA in other ethnic groups (i.e. Pacific) with higher metabolic disease risk. Our results may suggest that PA is more effective at improving plasma insulin concentrations and CRP in one ethnic group when compared to another but it may depend on baseline plasma insulin concentrations and CRP levels. These findings reiterate the importance of including ethnic minorities in research.

Importantly, results in this thesis show that positive associations between PA, body composition and markers of metabolic health can be observed even when participating in low levels of PA. Involvement in PA of any duration and intensity should be strongly promoted, as it is likely beneficial to all women, in particular women with obesity. This research suggests it would likely be more feasible to recommend shifts from SB to LPA than from LPA to MVPA (or from SB to MVPA). Part of the WHO strategy for reducing overweight and obesity is to reduce SB and results of this research support this (World Health Organization). Additionally, since increased sedentary bout length was adversely associated with BF%, our study suggests interventions aimed at reducing SB should also consider including decreasing bout length of SB. Results of this research support that more research is required before clear quantitative guidelines for SB can be determined. Results from this research also suggest it may be appropriate for these SB guidelines as well as PA guidelines to differ depending on ethnic group i.e. Pacific women's body composition and metabolic health did not appear to be

associated with SB whereas higher total PA and MVPA had positive associations with body composition and metabolic health. In contrast among NZE women higher SB had negative associations with both BF% and metabolic health markers. Therefore public health guidelines for Pacific women may require more emphasis on encouraging PA of all intensities whilst public health guidelines for NZE women could put equal emphasis on reducing SB and encouraging PA.

This is the first time that ethnic-specific associations between increased PA or decreased SB and the gut microbiota have been reported among NZ women. It is was interesting to find significant associations between increased PA, decreased SB and bacteria with the potential to produce butyrate, in this population. However more needs to be known about the mechanisms driving associations between PA, SB and the gut microbiota to enable this research and future research in this area to be interpreted more clearly.

Increased PA and reduced SB both have the potential to significantly improve the long-term health of NZ women through reduced BF% and consequently prevalence of obesity as well as reduced metabolic disease risk. By contributing to our understanding of how PA contributes to health and how this differs between Pacific and NZE women, the findings in this thesis will assist in shaping the design of future research and public health interventions aimed to reduce the overall prevalence and inequalities in obesity and metabolic health. This research reiterates that government agencies, such as the NZ Ministry of Health, must consider deprivation when developing these programmes, policies and guidelines. Ideally, this will eventually lead to the development and provision of public health guidelines but also environments that make engaging in regular PA achievable and sustainable for all people.

8.5 Key findings

- Pacific women participated in significantly less MVPA when compared to NZE women.
- Pacific women with a low-BF% (a minority ethnic group within NZ) participated in higher levels of SB when compared to NZE women.
- Pacific women were significantly more deprived with compared to NZE women.
- Pacific women's fasting plasma insulin concentrations were almost two-fold higher than their NZE counterparts ($p \leq 0.05$) and they also had significantly higher HbA1c levels ($p \leq 0.05$).

- Plasma glucose concentrations were higher among Pacific women with a high-BF% than for NZE with a high-BF% (p<0.05). A
- NZE women with a high-BF%, had increased CRP and LDL when compared to Pacific women (p<0.05).
- For both Pacific and NZE women, every 10-minute increase in MVPA was associated with 8% lower fasting plasma insulin concentration.
- A significant inverse association between increasing total PA and fasting plasma insulin concentration among Pacific women; every 100 cpm increase in total PA was associated with a 9% decrease in fasting plasma insulin concentration; no significant association was observed in NZE women.
- Among NZE women every 100 cpm increase in total PA and 10-minute increase in MVPA was significantly associated with a 9% and 10% decrease (p<0.05) in fasting CRP concentration, respectively; no significant association was observed in Pacific women.
- Higher total PA and heart rate for NZE women: every 100 cpm increase in daily PA was associated with a lower resting heart rate of 1.4 beats per minute (p<0.05).
- Every additional 10 minutes spent in MVPA was significantly associated with a lower heart rate among Pacific and NZE women's (1.8 and 1.2 beats/minute, respectively)
- Among NZE women, decreased time spent in sedentary was associated with lower gynoid fat (p<0.05), and lower weighted median sedentary bout length was associated with lower BF% (gynoid fat, total body, trunk, android and visceral fat (p<0.05)). No associations between SB and body composition were found among Pacific women.
- Among NZE every one-hour increase in sedentary time was associated with a 14% higher CRP (p<0.05). No associations between SB and metabolic markers were found among Pacific women.
- A decreased odds of meeting the PA guidelines among women who were in the lowest quartile of fibre intake compared to the top three quartiles (NZE women OR 0.66, p=0.021 and all women OR 0.72, p=0.008).

- A decreased odds of meeting the PA guidelines among women who were in the lowest quartile of polyunsaturated fat intake when measured as a % of total energy intake (Pacific women OR 0.67, p=0.030 and all women OR 0.76, p=0.027)
- Among NZE women, every one SD increase in total PA (197 cpm/day) was associated with 36.3% higher relative abundance of *Erysipelotrichaceae* (p=0.031) and 37.9% lower relative abundance of *Verrucomicrobiaceae* (p=0.029). Additionally, every one SD increase in SB (1.45 hours/day) was associated with a 28% lower relative abundance of *Erysipelotrichaceae* (p=0.030).
- For every 1 SD increase in total PA NZE women were 1.623 times more likely to be characterised by enterotype 1 and 41.6% less likely to be characterised by enterotype 3. Additionally, every 1 SD increase in SB, NZE women were 39.4% less likely to be characterised by enterotype 1 and 1.829 times more likely to be characterised by enterotype 3.
- Among Pacific women, every 1 SD increase in MVPA was associated with 22.8% lower (p=0.034) *Firmicutes:Bacteroidetes* ratio, whereas every 1 SD increase in NZE women's total PA was associated with 23.1% higher *Firmicutes:Bacteroidetes* ratio (p=0.031).

8.6 Public health recommendations

- Involvement in PA of any duration and intensity should be strongly promoted, as it is likely beneficial to all women, in particular women with obesity or poor metabolic health profile.
- Continue to promote the reduction of SB in all ethnic groups.
- Recommendations and research aimed at reducing SB should also consider strategies for decreasing bout length of SB.
- Government agencies, such as the NZ Ministry of Health, must consider deprivation in the programming, policy making and promotion of healthful diet and PA patterns.
- It may be more effective to tailor PA and SB promotion and recommendations to specific ethnic groups.

8.7 Strengths and limitations, reflection of methods and design

Strengths and limitations specific to each chapter have already been discussed, however there are also some wider strengths and limitations, relevant to all four research chapters. These should be taken into consideration when interpreting the overall findings of this research.

8.7.1 Strengths

- The study is strengthened by its relatively large population size.
- The objective measurement of PA is another strength to this study. The accelerometer data collection, in a free-living setting, across a full 24-hour period and over a week, was important given the variability in activities performed within a day and across a week. This method may have improved wear-time compliance. It also allowed for the objective measurement of sleep onset and wake times, without relying on algorithms to predict this data, meaning the objective nature of the data was not compromised.
- A 5-day non-consecutive estimated food record was utilised in this study. Each participant completed the food record on both week days and at least one weekend day. Food records were also reviewed in a one on one interview with each participant and a NZ Registered Dietitian. Dietary intake data obtained using this method was robust.
- A strength was the use of DXA for estimating BF%, as it is well recognised for its precision and accuracy (Prior et al., 1997). Whilst there is no universally agreed upon definition of obesity in terms of BF% (Oliveros et al., 2014), a range of BF% cut-off points have been used for women, varying between 30 to 37% (Oliveros et al., 2014). The chosen cut-point for categorising high vs. low BF% (35%) was confirmed after carrying out analysis within our population comparing BF% with BMI. Specifically, scatter plots, graphing BMI vs. BF% with a line of best fit indicated using 35% BF as our cut-point. Using this cut point only five participants with a BMI <25 kg/m^2 were classified in the $>35\%$ BF group, suggesting potential misclassification in terms of BMI was minor. This also supports the use of BMI for future obesity research as both BMI and BF% demonstrate very similar results when classifying participants as obese. This is particularly relevant if funding does not allow the use of costly DXA scans.
- This study overcame limitations of other studies in this area of PA and the gut microbiota, by including the confounding factors of diet and sleep in statistical analysis

(Barton et al., 2018, Durk et al., 2019, Mörkl et al., 2017, Estaki et al., 2016, Clarke et al., 2014b).

8.7.2 Limitations

- The PROMISE study is cross-sectional, with all of the inherent limitations of this design. The direction of causality cannot be determined. Additionally, the described patterns, for example PA, diet, metabolic markers and the gut microbiota only provide a snapshot in time. None-the-less, cross-sectional studies provide important insights into the associations and relationships that are not easily revealed by other designs.
- The study population was not a general representative sample of NZE and Pacific women living in NZ. Participants were selectively recruited based on age, BMI and ethnicity, were pre-menopausal, and were free of chronic disease. Our results are only applicable to this population.
- All measurements were collected in an urban setting in the Auckland, NZ metropolitan area, it is therefore not possible to expand the findings in this thesis to individuals living in a rural setting. Mode of transport, access to PA facilities, and types of food consumed could be different in an urban versus rural setting, influencing PA, SB and dietary choices. However, residential areas within the Auckland metropole do vary considerably in regard to socio-economic status, which was evident in this study (Otara North; NZDep2013 decile 10.00, Herne Bay NZDep2013 decile 1.00).
- Travel to the research centre was a large barrier for some potential participants, limiting their ability to take part in the research. This is despite offering compensation in the form of a petrol or supermarket voucher for travel costs and time.
- Individuals who choose to take part in health research are often interested in health and because of this, potentially live healthy lifestyles compared to the general public. This may have biased our results.
- Unfortunately, information was not gathered about where in the Pacific the Pacific women in this study identify with. It is increasingly recognised that significant heterogeneity exists even within these comparatively narrow ethnic groups for example religious beliefs, migrant generation status, level of deprivation, education level and language (Pasefica Proud). All these factors and other factors may impact levels of PA and SB and diet. Future studies should collect this information.

- The wider PROMISE study set out to recruit a) Pacific women with both parents identifying as Pacific and b) Pacific women with a BMI of <25 kg/m^2. Researchers struggled to recruit these women. The criteria was subsequently extended to include a) Pacific women who had one parent who identified as Pacific and b) BMI <27 kg/m^2, so long as the participating Pacific woman identified as Pacific ethnicity (the majority of this group still fell within the original criterial of <25 kg/m^2).
- Accelerometer cut-points have been validated in European populations therefore may not be applicable to different ethnic groups, including Pacific. Also, there are no female specific, validated cut-points.
- This research only investigated the whole diet in terms of energy, macro- and micronutrients. Future research should consider additional analysis to focus on food choices as well as nutrients to better determine the relationship with PA and SB.

8.8 Future perspectives

Future research needs to depend and expand on the findings in this thesis.

- Longitudinal investigations over five plus years will assist in disclosing possible long-term associations between PA, SB and metabolic health and the gut microbiota composition later in life. Clinical trials or intervention studies will on the other hand shed light on the casual relationships (in combination with observational research). Both types of research are needed for providing sound, valid scientific evidence to progress making public health PA and SB recommendations in this population.
- The 24-hour day is finite, therefore the time spent in all movement behaviours is co-dependent (sleep, SB, light intensity PA and MVPA); Future studies should consider using more sophisticated statistical analysis (e.g. compositional analysis) that would account for this (Chastin et al., 2015).
- Exploring the possibility of off-site data collection. Pacific women in this study predominantly resided in South Auckland and were required to travel >1 hour to the study clinic, a large barrier to participation. Future research strategies should focus on delivering the research within the community where it will be easily accessible for all.
- Dietary pattern analysis could be considered to assess the association of the whole diet in relation to PA and SB.

- Focusing on a different approach such as a food-based approach e.g. dietary patterns, may be beneficial to further explore and develop a deeper understanding of the relationship between food choice and PA.
- For future improvements in objective measures of PA and SB, gender- and ethnic-specific accelerometer cut-points could be developed and validated.
- In view of the important role of PA in promoting health, solutions to break down barriers to PA among groups of varying deprivation should be examined. This may include assessing the impact of providing free calisthenic equipment available in public parks in areas of low socioeconomic status.
- Research needs to continue to investigate associations between PA and the gut microbiota in non-athletic populations. More needs to be known about the mechanisms driving associations between PA, SB and the gut microbiota.

8.9 References

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Appendices

1. Publications (published), conference presentations and abstracts
2. PROMISE study information sheet and consent form
3. Demographic questionnaire
4. Accelerometer information sheet
5. Food record
6. Pittsburg sleep quality index (PSQI)
7. Faecal collection information

Appendix 1

List of papers and conference presentations

The following publications were written during the PhD candidature.

Peer-review publications

Slater, J., Kruger, R., Douwes, J., O'Brien, W.J., Corbin, M., Miles-Chan, J.L. and Breier, B.H., 2021. Objectively measured physical activity is associated with body composition and metabolic profiles of Pacific and New Zealand European women with different metabolic disease risks. *Frontiers in Physiology*, *12*, p.684782.

Publications under review or to be submitted

Joanne Slater, Jennifer L. Miles-Chan, Jeroen Douwes, Marine Corbin, Carol Wham, Bernhard H. Breier, Rozanne Kruger. Objectively measured sedentary behaviour and metabolic health in Pacific and New Zealand European women.

Joanne Slater, Jennifer L. Miles-Chan, Jeroen Douwes, Marine Corbin, Renall Nikki, Carol Wham, Bernhard H. Breier, Rozanne Kruger. Exploring associations between physical activity and nutrient intake among Pacific and New Zealand European women.

Joanne Slater, Gerald Tannock, Jennifer L. Miles-Chan, Jeroen Douwes, Marine Corbin, Renall Nikki, Carol Wham, Bernhard H. Breier, Rozanne Kruger. Is objectively measured physical activity and sedentary behaviour associated with the gut microbiota among Pacific and New Zealand European women?

Additional peer-reviewed publications completed during the candidature

Kindleysides, S., Kruger, R., Douwes, J., Tannock, G.W., Renall, N., Slater, J., Lawley, B., McGill, A.T., Brennan, N., Manukia, M. and Richter, M., Tupai-Firestone, R., Signal, TL., Gander, P., Stannard, SR., Breier, BH. 2019. Predictors linking obesity and the gut microbiome (the promise study): protocol and recruitment strategy for a cross-sectional study on pathways that affect the gut microbiome and its impact on obesity. *JMIR research protocols*, *8*(8), p.e14529.

Oral presentations

Slater J, Kruger R, Renall N, Richter M Corbin M, O'Brien W, Signal TL, Breier BH. Physical Activity and Metabolic Health in New Zealand European and Pacific Women from the PROMISE Study. Nutrition Society of NZ conference, 2018. (Awarded best student presenter).

Poster presentations

Slater J, Miles-Chan JL, Douwes J, Corbin M, Wham C, Breier BH, Kruger R. Is objectively measured sedentary behaviour associated with body composition of Pacific and New Zealand European women? Australia New Zealand Obesity Society Conference, poster presentation, July 2021.

frontiers
in Physiology

ORIGINAL RESEARCH
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Objectively Measured Physical Activity Is Associated With Body Composition and Metabolic Profiles of Pacific and New Zealand European Women With Different Metabolic Disease Risks

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Objective: To assess associations between physical activity (PA), body composition, and biomarkers of metabolic health in Pacific and New Zealand European (NZE) women who are known to have different metabolic disease risks.

Methods: Pacific (n = 142) or NZE (n = 162) women aged 18–45 years with a self-reported body mass index (BMI) of either 18.5–25.0 kg·m^{-2} or ≥30.0 kg·m^{-2} were recruited and subsequently stratified as either low (<35%) or high (≥35%) BF%, with approximately half of each group in either category. Seven-day accelerometery was used to assess PA levels. Fasting blood was analysed for biomarkers of metabolic health, and whole body dual-energy X-ray absorptiometry (DXA) was used to estimate body composition.

Results: Mean moderate-to-vigorous physical activity (MVPA; min·day^{-1}) levels differed between BF% ($p < 0.05$) and ethnic ($p < 0.05$) groups: Pacific high- 19.1 (SD 15.2) and low-BF% 26.3 (SD 15.6) and NZE high- 30.5 (SD 19.1) and low-BF% 39.1 (SD 18.4). On average Pacific women in the low-BF% group engaged in significantly less total PA when compared to NZE women in the low-BF% group (133 cpm); no ethnic difference in mean total PA (cpm) between high-BF% groups were observed: Pacific high- 607 (SD 185) and low-BF% 598 (SD 168) and NZE high- 674 (SD 210) and low-BF% 731 (SD 179). Multiple linear regression analysis controlling for age and deprivation showed a significant inverse association between increasing total PA and fasting plasma insulin among Pacific women; every 100 cpm increase in total PA was associated with a 6% lower fasting plasma insulin; no significant association was observed in NZE women. For both Pacific and NZE women, there was an 8% reduction in fasting plasma insulin for every 10-min increase in MVPA ($p \leq 0.05$).

Appendix 2

MASSEY UNIVERSITY
COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

PROMISE STUDY

(PRedictors linking Obesity and gut MIcrobiomE)

INFORMATION SHEET

We are looking for women aged between 18 and 45 years of age to take part in the PROMISE study. We want to understand how the bacteria in our gut are affected by diet, physical activity, sleep and taste perception, and how this is linked to health and a healthy body weight. Please read this information sheet carefully before deciding whether or not to participate.

What is this research about?

Here in New Zealand we are experiencing an epidemic of obesity and metabolic diseases, such as diabetes. A key contributing factor is our diets and the types of food we eat. Food has a large impact on the number and types of bacteria in our bowel (gut microbiome). There are at least 1000 different types of bacteria. It is thought that these bacteria play a very important role in our health, and may be contributing to obesity in some people. In this study we will investigate how diet, taste perception, sleep and physical activity affect the gut microbiome. This new knowledge will help us understand obesity and how best to prevent it.

Who are we looking for?

We are looking for women to participate in this study. To take part in this study you need to:

- Be between 18 and 45 years of age

- Be of NZ European ethnicity **OR** of Pacific ethnicity
- Not be pregnant or breastfeeding
- Not have any chronic diseases such as heart disease, diabetes or cancer
- Not have any food-allergies
- Not be following a severely restricted diet

What is involved in the study?

If you decide to take part in the study, you will be asked to complete a screening questionnaire (online or over the phone) that will assess your eligibility. If you meet these eligibility criteria you will be invited to take part in the study, which includes 2 visits to the Massey University Human Nutrition Research Unit in Albany and collection of data at home.

Visit 1 (approximately 2.5 hours)

You will have to visit the Human Nutrition Research Unit early in the morning (between 7.30 and 8:30am), without having eaten breakfast. The visit will take approximately 2.5 hours. On the day we will first explain to you what is involved in the study and answer all your questions. We will then ask you to sign a consent form.

The following procedures will take place during this visit:

- Your height, weight and body composition will be measured.
- A blood sample will be taken from a vein in your arm by a trained person (a phlebotomist). For this blood sample you need to have fasted overnight. **You should not eat or drink anything (other than water) from 10pm the previous evening until after the blood sample has been taken.** We will use the blood samples to measure cholesterol and glucose levels, markers of inflammation and small molecules related to fat usage and storage.
- You will be asked to taste a few sweet, fat and bitter solutions by sipping a mouthful (10mL or 2 teaspoons), swirling it in your mouth and swallowing it, and then rating your preference on a scale.

- A buffet-style continental breakfast will be provided prior to further measures to ensure that you break your fasted state.
- You will also be asked to complete some questionnaires regarding your diet and sleep patterns.
- We will ask you to provide a small urine sample.

At home between Visits 1 & Visit 2

You will be asked to:

- Wear an Accelerometer (a small device similar to a step-counter that you wear around your waist to measure physical activity), and an Actiwatch (a watch worn on your wrist which records your sleep patterns) for 7 days. The accelerometer and watch will be provided by the researchers and you will receive detailed instructions on how to use them.
- Record your physical activity for 7 days while you are wearing the small devices above.
- Record your food intake for 5 days while you are wearing the small devices above.
- Provide two small faecal (stool) samples and bring these to Visit 2. It is important that these two small stool samples are frozen (in the containers provided by us) immediately after collection in your freezer. These samples allow us to determine the bacteria (microbes) in your gut.

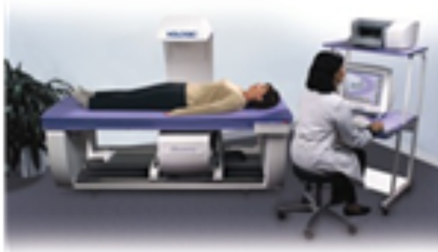
Visit 2 (approximately 2 hours)

You will be asked to visit the Human Nutrition Research Unit during the day. For this visit we ask that you eat as usual before coming as no food will be provided during Visit 2. The visit will take about 2 hours.

At this visit:

- You will bring back all your devices and the stool samples and your physical activity and food records;

- An in-depth interview will be conducted regarding your data collection tasks including devices and food recording by the research staff.
- Your weight and height will be measured.
- Your blood pressure will be measured.
- You will undergo a full body composition assessment including waist, hip and abdominal height measurements as well as a whole body scan using a DXA machine. These measurements will take place in a private, enclosed room.
- You will also be asked to complete some questionnaires regarding your diet and activity patterns.



DXA machine

What are the benefits and risks of taking part in this study?

There will be no charges made for any of the tests that you undertake. You will receive information regarding your own individual measurements (blood results including blood sugar, cholesterol, blood pressure, % body fat and BMI) and an explanation of the data. In recognition of your participation, you will receive a $100 supermarket or petrol voucher at the end of visit 2. You will also receive a brief report summarising the main findings of the project via mail or e-mail after analysis of the data has been completed. This is also a chance for you to receive some information about your current health status. In addition, this information will help us to provide better recommendations for preventing obesity.

Some people may have a fear of having a blood sample taken or experience discomfort when the blood samples are taken. Occasionally a slight bruise will result. We will take every

measure to ensure that you are comfortable and respected. You may also be accompanied by a support person if required.

With the DXA body composition scan you will be exposed to a very low dose of radiation, unlikely to cause any harm. The total dose of radiation will be less than the amount of radiation you are exposed to during a trans-Tasman flight.

Project procedures

Sample Handling and Storage

Samples will be stored in a secure laboratory freezer at the Human Nutrition Research Unit until completion of the study – for a maximum of 10 years. Samples will be analysed by fully-accredited laboratories, either in NZ or overseas, depending on the type of analysis being done. The data will be used only for the purposes of this project and no individual will be identified. Only the investigators and administrators of the study will have access to personal information and this will be kept secure and strictly confidential. Participants will be identified only by a study identification number to ensure anonymity and confidentiality of these samples. Following analysis samples will be destroyed following usual procedures, however, these blood samples can be returned to you upon request.

Data Handling

Results of this project may be published or presented at conferences or seminars. No individual will be able to be identified. At the end of this study the list of participants and their study identification number will be disposed of. Any raw data on which the results of the project depend will be retained in secure storage for 10 years, after which it will be destroyed.

Genetic analysis

Each person has a DNA make-up (their genes) which is different from that of everybody else – except in the case of identical twins. As part of this study we will be performing some DNA-based measures on blood samples that you provide us with. This will allow us to investigate

possible links between the gut microbiome and genetic makeup, in particular, links that may relate to diet, body composition, sleep patterns, taste perception and physical activities. This may further our understanding of specific risk factors for obesity. The genetic or DNA-based measures that we will be performing will be research-based and will not have any medical significance for you. Therefore you will not receive the results of these genetic measures as any information gathered will be purely theoretical. As with all other aspects of the study, ID numbers will be used so that your samples are not identifiable and all information will be kept confidential and will only be used for the specific purposes of this study.

Consenting to allow DNA-based measures to be performed on your samples is entirely optional. If you do not wish to participate in this aspect of the research please indicate this on your consent form. You may withdraw your consent to the use of your sample in this research at any time and your samples will be destroyed.

Who is funding the research?

This research is funded by the Health Research Council (HRC) of New Zealand (Grant #15/273).

Participant's rights

You are under no obligation to accept this invitation. If you decide to participate, you have the right to:

- decline to answer any particular question;
- withdraw from the study at any time;
- ask any questions about the study at any time during participation;
- provide information on the understanding that your name will not be used unless you give permission to the researcher;
- be given access to a summary of the project findings when it is concluded.

Project contacts

If you have any further questions or concerns about the project, either now or in the future, please contact the research team on 09 212 7013 or 021 082 74425 or e-mail promise@massey.ac.nz or website www.massey.ac.nz/promise.

The lead researchers for this study are Professor Bernhard Breier and Associate Professor Rozanne Kruger. If you have any concerns please contact Bernhard [b.breier@massey.ac.nz, (09) 213 6652] or Rozanne [r.kruger@massey.ac.nz, (09) 213 6661]

Committee approval statement

This project has been reviewed and approved by the Health and Disability Ethics Committee, Southern Region, Ethics Reference 16/STH/32. If you have any concerns about the conduct of this research, please contact the Southern Health and Disability Ethics Committee on 0800 438442 or hdecs@moh.govt.nz

Compensation for injury

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic and your claim will be assessed by ACC in accordance with the Accident Compensation Act 2001. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. If your ACC claim is not accepted you should immediately contact the researchers.

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Consent Form for Participants

I have read the Information Sheet* and have had the details of the study explained to me. My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.

I agree to participate in this study under the conditions set out in the Information Sheet*. This consent form will be held for a period of ten (10) years.

☐ Yes ☐ No

DNA analysis will be performed on parts of the blood samples obtained. Do you consent to parts of your samples being used for DNA analysis?*

☐ Yes ☐ No

The findings of this study may be the basis of further investigations by our team. Would you be happy for us to contact you in future about research related to this study?

☐ Yes ☐ No

*Please see the study Information Sheet for more details

Signature: .... **Date:** ....

Full Name (printed) ....

Appendix 3

Subject Number:

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Health and Demographics Questionnaire

What is your first language?

English ☐

Other ☐ Please state:___________________________________

Do you have children? Yes ☐ No ☐

If yes,
How many children do you have? ______________________

What are their age(s)? ________________________________

When was your youngest child born? __ / __ / ____ (Day/Month/Year)

What is the highest level of education you have received?

Primary School ☐

Secondary School (College e.g. school certificate, Bursary, NCEA level 1-3) ☐

Trade certificate or diploma ☐

University or other tertiary education (e.g. postgraduate diploma and certificate, bachelor's degree, Masters, PhD) ☐

Other (please state) ☐ ______________________

What would be the total income that the <u>household</u> received from <u>all sources</u> before tax has been taken out in the last 12 months?

Loss	☐	\$30,001 - \$40,000	☐
Zero	☐	\$40,001 - \$50,000	☐
\$1 - \$5,000	☐	\$50,001 - \$70,000	☐
\$5,001 - \$10,000	☐	\$70,001 – 100,000	☐

$10,001 - $15,000	☐	$100,000 – or more	☐
$15,001 - $20,000	☐	I don't want to answer	☐
$20,001 - $30,000	☐		

During the past 5 years, what was your main occupation or job title? (eg teacher, veterinarian, dairy farmer, librarian, social worker, housekeeping, etc.)?

Please be as specific as possible
Fill in 'unemployed' if you were unemployed for most of the past 5 years

Occupation **mm/yy** **mm/yy** **Still working**

1 ____ _ from ___/___ to ___/___ ☐

2 ____ from ___/___ to ___/___ ☐

3 ____ from ___/___ to ___/___ ☐

4 ____ from ___/___ to ___/___ ☐

5 ____ from ___/___ to ___/___ ☐

In your current profession what was the main activity of the Company or Organisation you worked for? (If currently unemployed, refer to your last recent employment)

Please be as specific as possible
Fill in 'not applicable' if you were unemployed for most of the time during the past 5 years or when your main activity was housekeeping

Please describe
1 ____
_

2 ____
_

3 ____
_

4 ____
_

5 ____
_

How many hours do you USUALLY work each WEEK? __________ hours

How many hours do you USUALLY work each DAY? __________ hours

What is your USUAL work pattern?

Please tick the box that best applies.

daytime with no shifts ☐

rotating shifts with nights ☐

rotating shifts without nights ☐

permanent nights ☐

irregular or variable ☐

other work pattern ☐ Please specify ____________________

If you work night shifts, how many do you work in a USUAL week? _______ nights out of 7

<u>Diet and Health</u>

Do you follow a specific diet? Yes ☐ No ☐

If yes, please specify the diet that you follow:____________________________________

____

_

If yes, do you follow this diet for health reasons? Yes ☐ No ☐

If no, why do you follow this diet?

____

____

Do you follow any diet for cultural or religious reasons? Yes ☐ No ☐

If yes, what type of diet do you follow? ______________________________________

____

____

Would you say you eat your meals

Quickly ☐ At a moderate pace ☐ Slowly ☐

When did your last period start? __ / __ / ____ (Day/Month/Year)

Are you pregnant? Yes ☐ No ☐

Do you have any surgical implants (eg pins in bones/teeth, pacemaker, cosmetic)?

Yes ☐ No ☐

Do you smoke cigarettes? Yes ☐ No ☐

If yes, approximately how many cigarettes per day: ______________________________

Do you drink alcohol? Yes ☐ No ☐

If yes, approximately how many standard drinks per week:__________ ____________

[1 standard drink = 1 glass of wine (100ml), 1 bottle/can of beer, 1 nip of spirits (45mL)]

Do you have any allergies? Yes ☐ No ☐

Please specify ____

____

Are you taking any form of medication, including traditional or homeopathic medicine and contraception? Yes ☐ No ☐

If yes, please specify the condition, the medication and the dosage in the table below.

Condition	Medication	Dosage	Frequency

Are you taking any probiotics or prebiotics? Yes ☐ No ☐

If yes please provide the brand name and variety ___________________________

Are you taking any form of supplements, including tablets or drinks? Yes ☐ No ☐

If yes, please tell us the name, brand and dosage of the supplements you are taking in the table below.

Supplement	Brand	Dosage	Frequency

By what method were you born?

☐ Cesarean

☐ Natural (vaginal)

☐ I don't know

What was your birth weight in pounds: __________

Please tell us how you found out about this study. Did you find out from:

☐ A friend?

☐ An email list?

If yes, what is the name of the email list?______________________________

☐ At an event?

If yes, which event?____

☐ Flyer on noticeboard?

If yes, where was the noticeboard?___________________________________

☐ The Fono medical clinic

☐

Other____

Would you like to receive a brief report summarizing the main findings of the project? Yes ☐ No ☐

Are you willing to be contacted in future research projects within the Massey University School of Food and Nutrition? Yes ☐ No☐

Appendix 4

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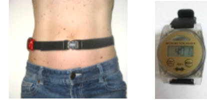
What is an ActiGraph device?



- An ActiGraph device is a motion sensor which measures your movement. We are providing you with two ActiGraph devices a watch and a hip belt. They are expensive pieces of equipment so it is important you take good care of then and return them on visit 2!

How do I wear and operate the ActiGraph devices?

Hip belt

- Your hip belt ActiGraph device is threaded onto an elastic belt on which it must remain.
- The elastic belt with the ActiGraph device on it should fit firmly around your hips (under your clothes).
- The ActiGraph device can be taken on and off using the clip fastener on the elastic belt.
- The ActiGraph device must be positioned on the **right hand side of your waist/hip**, just above your hip bone and in line with your armpit as shown in Fig 1 and 2.

Correct

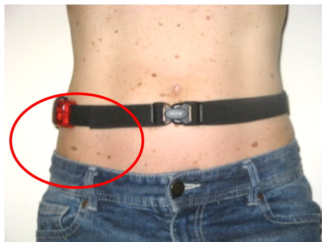


Fig 1 Right hip/waist

Correct

Fig 2 Right side

- The ActiGraph device must be worn on the **right waist/hip**, not in the centre of your body as in Fig 3.
- The elastic belt must **not sag or be twisted** (Fig 2) and must be **FIRM** around your hips (Fig 1).
- Make sure the ActiGraph device is in an upright position and is not tilted as in Fig 4.

Incorrect

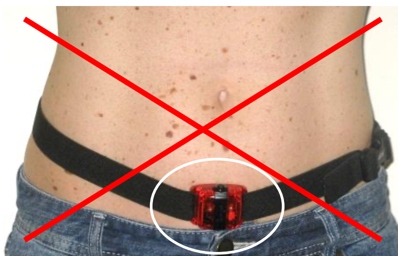


Fig 3 Not in centre of

Incorrect

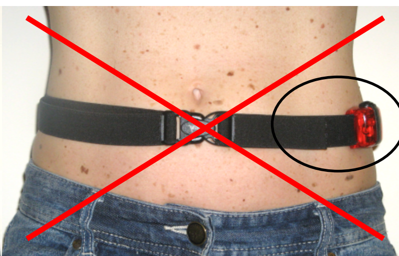


Fig 4 Not on left side

Incorrect

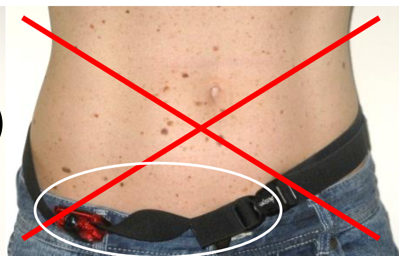


Fig 5 Not loose, must be firm around waist/hip

Please DO NOT wear the ActiGraph device in an incorrect place or position.
ActiGraph devices worn incorrectly (as in Fig 3, 4 or 5) will NOT work properly.

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- If possible, wear the **hip** belt ActiGraph device under clothing at all times.
- Reposition the hip belt ActiGraph device above your right hip first thing in the morning as soon as you wake up and any time during the day when it slips or moves.
- The **hip** belt ActiGraph device must remain on the elastic belt at all times and should be put on and taken off using the clip fastener.

Watch

- Wear the watch on your non-dominant wrist (the hand you don't write with).
- It is important that once you begin wearing the watch you do not change wrists as this may significantly change the information that we get from the watch.
- The watch should be attached reasonably firmly so that it does not move about on your wrist. If it does move about tighten the strap slightly.
- We cannot tell what you are doing from the watch data. We can only tell whether you are moving or not.
- The button on the face left hand side is a marker (event) button. If you push this a small mark will appear on the data output. It does not stop or start the watch. The watch will keep going the entire time you are wearing it.
- **We would like you to push the marker (event) button when you start trying to sleep and again when you stop trying to sleep. Please do this whenever you intend to sleep for 10 minutes or longer.**

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When do I wear the ActiGraph devices?

You need to wear your ActiGraph devices (hip belt and watch) for **XXXXdays** ....

Starting today when you receive the device

Finishing any time after midday on ____

- Please wear the ActiGraph devices **ALL THE TIME (**except for showering or water based activities like swimming as they are not waterproof), including to bed and during sport or exercise.

- The ActiGraph devices may be taken off **ONLY** when doing **water based activities** where they would get completely wet or submerged (e.g., swimming or showering). The devices should be worn for other water activities such as kayaking where it won't get completely wet.

- **Remember,** if the ActiGraph devices are removed at any time (e.g. swimming, showering):
  - Put them back on as soon as you have finished.
  - Be sure to **record in your diary** when the ActiGraph devices were removed and put back on.

What information do I need to record in the PROMISE Study Diary?

- Sleep
- Physical activity
- ActiGraph (hip belt and watch) removal

Section 1. Physical activity and Actigraph removal

- We need to know when the ActiGraphs were worn and not worn during each day (e.g., taken off for a shower).

- **If the ActiGraph devices are taken off,** please note the time when they were removed and replaced and what activity you were doing during that time.

- We'd also like to know about any activities you do with the aim of improving your health or fitness, or for sport, e.g. if you go for a walk, or to the gym or bike riding. For these activities, record for each day, the start time, length of time you did the activity, and the intensity (light, moderate or vigorous as explained on your diary).

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Section 2. Sleep

- The sleep diary is set out so that each line represents 24 hours, from midnight to midnight on one day.
- We are interested in **any** sleep that is 10 minutes or longer. It does not matter whether this is during the day or during the night.
- The information that is important to us are the times that you **begin trying to sleep** and when you **finish trying to sleep** after any sleep that is **10 minutes or longer.**
- When you are about to **start trying to sleep:**

  a) Start (S) is the time when you start trying to sleep. Some people may get into bed and read etc, but we do not need to know this, we only need to know when you start trying to go to sleep.

  b) Mark it on the timeline with an arrow and write 'S' along with the time in hours and minutes above or under it.

- When you have **finished trying to sleep:**

  a) End (E) is when you wake up and are no longer trying to sleep. At this time you may either get out bed or begin to read etc, but you are no longer trying to sleep.

  b) Mark it on the timeline with an arrow and write 'E' along with the time in hours and minutes above or under it.

- **Start** and **End** are the times we would like you to push the marker button on the ActiGraph Watch.
- If you wake up during your sleep to get a drink, go to the toilet etc, you do not need to write anything in the sleep/duty diary. If you get up for **more than 10 minutes,** then please treat any later sleep as a new sleep period.

If you have any queries, questions or concerns whilst wearing the ActiGraph, please don't hesitate to contact us on ________________ or promise@massey.ac.nz

Appendix 5

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PROMISE Study

5 Day Food Record

Thank you very much for taking part in the PROMISE Study. We are extremely grateful for your time, effort and commitment!

If you have any questions, please contact PROMISE staff on:
414 0800 (extn 49013)   email: promise@massey.ac.nz

All information in this diary will be treated with the strictest confidence. No one outside the PROMISE study will have access to this data.

Please bring this food diary with you to visit 2 at the Nutrition Laboratory

What to do?

- Record all that you eat and drink on the following dates.

____
____
____
____
____

- If possible record food at the time of eating or just after – try to avoid doing it from memory at the end of the day.

- Include all meals, snacks, and drinks, even tap water.

- Include anything you have added to foods such as sauces, gravies, spreads, dressings, etc.

- Write down any information that might indicate **size or weight** of the food to identify the portion size eaten.

- Use a new line for each food and drink. You can use more than one line for a food or drink. See the examples given.

- Use as many pages of the booklet as you need.

Describing Food and Drink

- Provide as much detail as possible about the type of food eaten. For example **brand names and varieties / types** of food.

General description	Food record description
Breakfast example – cereal, milk, sugar	1 cup Sanitarium Natural Muesli 1 cup Pam's whole milk 1 tsp Chelsea white sugar
Coffee	1 tsp Gregg's instant coffee 1 x 200ml cup of water 2 Tbsp Meadow fresh light green milk
Pasta	1 cup San Remo whole grain pasta spirals (boiled)
Pie	Big Ben Classic Mince and Cheese Pie (170g)

- Give details of all the **cooking methods** used. For example, fried, grilled, baked, poached, boiled…

General description	Food record description
2 eggs	2 size 7 eggs fried in 2tsp canola oil 2 size 6 eggs (soft boiled)
Fish	100g salmon (no skin) poached in 1 cup of water for 10 minutes

- When using foods that are cooked (eg. pasta, rice, meat, vegetables, etc), please record the **cooked portion** of food.

General description	Food record description
Rice	1 cup cooked Jasmine rice (cooked on stove top)
Meat	90g lean T-bone steak (fat and bone removed)
Vegetables	½ cup cooked mixed vegetables (Wattie's peas, corn, carrots)

- Please specify the **actual amount of food eaten** (eg. for leftovers, foods where there is waste)

General description	Food record description
Apple	1 x 120g Granny Smith apple (peeled, core not eaten – core equated to ¼ of the apple)
Fried chicken drumstick	100g chicken drumstick (100g includes skin and bone); fried in 3 Tbsp Fern leaf semi-soft butter

- **Record recipes** of home prepared dishes where possible and the proportion of the dish you ate. There are blank pages for you to add recipes or additional information.

Recording the amounts of food you eat

It is important to also record the quantity of each food and drink consumed. This can be done in several ways.

- By using household measures – for example, cups, teaspoons and tablespoons. eg. 1 cup frozen peas, 1 heaped teaspoon of sugar.

- By weight marked on the packages – eg. a 425g tin of baked beans, a 32g cereal bar, 600ml Coke

- For bread – describe the size of the slices of bread (eg. sandwich, medium, toast) – also include brand and variety.

- Using comparisons – eg. Meat equal to the size of a pack of cards, a scoop of ice cream equal to the size of a hen's egg.

- Use the food record instructions provided to help describe portion sizes.

General description	Food record description
Cheese	1 heaped tablespoon of grated cheese 1 slice cheese (8.5 x 2.5 x 2mm) 1 cube cheese, match box size Size 10B grated cheese,

- If you go out for meals, describe the food eaten in as much detail as possible.

- ***Please eat as normally as possible - don't adjust what you would normally eat just because you are keeping a diet record and be honest! Your food record will be identified with a number rather than your name.***

Example day

Time food was eaten	**Complete description of food (food and beverage name, brand, variety, preparation method)**	**Amount consumed (units, measures, weight)**
7:55am	Sanitarium weetbix	2 weetbix
" "	Anchor Blue Top milk	150ml
" "	Chelsea white sugar	2 heaped teaspoons
" "	Orange juice (Citrus Tree with added calcium – nutrition label attached)	1 glass (275 ml)
10.00am	Raw Apple (gala)	Ate all of apple except the core, whole apple was 125g (core was ¼ of whole apple)
12.00pm	Home-made pizza (recipe attached)	1 slice (similar size to 1 slice of sandwich bread, 2 Tbsp tomato paste, 4 olives, 2 rashers bacon (fat removed), 1 Tbsp chopped spring onion, 3 Tbsp mozzarella cheese)
1.00pm	Water	500ml plain tap water
3.00pm	Biscuits, chocolate covered Girl Guide biscuits	6 x (standard size)
6.00pm	Lasagne	½ cup cooked mince, 1 cup cooked Budget lasagne shaped pasta , ½ cup Wattie's creamy mushroom and herb pasta sauce, ½ cup mixed vegetables (Pam's carrots, peas and corn), 4 Tbsp grated Edam cheese
6.30pm	Banana cake with chocolate icing (homemade, recipe attached)	1/8 of a cake (22cm diameter, 8 cm high), 2 Tbsp chocolate icing
" "	Tip Top Cookies and Cream ice cream	1 cup (250g)
7.30pm	Coffee	1 tsp Gregg's instant coffee 1 x 300ml cup of water 2 Tbsp Meadow fresh blue top milk 2 tsp sugar

Date________________________________ DAY 1

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed

Date________________________________ **DAY 1 (continued)**

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed

Date________________________________ DAY 1 (continued)

Time food was eaten	Complete description of food (food and beverage name, brand, variety, preparation method)	Amount consumed

Recipes (Day 1)

Appendix 6

Name:____ Date: _________________

Pittsburgh Sleep Quality Index (PSQI)

Instructions: The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. **Please answer all questions.**

1. During the past month, what time have you usually gone to bed at night? ___________________
2. During the past month, how long (in minutes) has it usually taken you to fall asleep each night? __________
3. During the past month, what time have you usually gotten up in the morning? ___________________
4. During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spent in bed.) ___________________

5. During the past month, how often have you had trouble sleeping because you…	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
a. Cannot get to sleep within 30 minutes				
b. Wake up in the middle of the night or early morning				
c. Have to get up to use the bathroom				
d. Cannot breathe comfortably				
e. Cough or snore loudly				
f. Feel too cold				
g. Feel too hot				
h. Have bad dreams				
i. Have pain				
j. Other reason(s), please describe:				
6. During the past month, how often have you taken medicine to help you sleep (prescribed or “over the counter”)?				
7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?				
	No problem at all	Only a very slight problem	Somewhat of a problem	A very big problem
8. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?				
	Very good	Fairly good	Fairly bad	Very bad
9. During the past month, how would you rate your sleep quality overall?				

	No bed partner or room mate	Partner/room mate in other room	Partner in same room but not same bed	Partner in same bed
10. Do you have a bed partner or room mate?				
	Not during the past month	Less than once a week	Once or twice a week	Three or more times a week
If you have a room mate or bed partner, ask him/her how often in the past month you have had:				
a. Loud snoring				
b. Long pauses between breaths while asleep				
c. Legs twitching or jerking while you sleep				
d. Episodes of disorientation or confusion during sleep				
e. Other restlessness while you sleep, please describe:				

Scoring the PSQI

The order of the PSQI items has been modified from the original order in order to fit the first 9 items (which are the only items that contribute to the total score) on a single page. Item 10, which is the second page of the scale, does not contribute to the PSQI score.

In scoring the PSQI, seven component scores are derived, each scored 0 (no difficulty) to 3 (severe difficulty). The component scores are summed to produce a global score (range 0 to 21). Higher scores indicate worse sleep quality.

Component 1: Subjective sleep quality—question 9

Response to Q9	Component 1 score
Very good	0
Fairly good	1
Fairly bad	2
Very bad	3

Component 1 score:_____

Component 2: Sleep latency—questions 2 and 5a

Response to Q2	Component 2/Q2 subscore
≤ 15 minutes	0
16-30 minutes	1
31-60 minutes	2
> 60 minutes	3

Response to Q5a	Component 2/Q5a subscore
Not during past month	0
Less than once a week	1
Once or twice a week	2
Three or more times a week	3

Sum of Q2 and Q5a subscores	Component 2 score
0	0
1-2	1
3-4	2
5-6	3

Component 2 score:_____

Component 3: Sleep duration—question 4

Response to Q4	Component 3 score
> 7 hours	0
6-7 hours	1
5-6 hours	2
< 5 hours	3

Component 3 score:_____

Component 4: Sleep efficiency—questions 1, 3, and 4

Sleep efficiency = (# hours slept/# hours in bed) X 100%

hours slept—question 4

hours in bed—calculated from responses to questions 1 and 3

Sleep efficiency	Component 4 score
> 85%	0
75-84%	1
65-74%	2
< 65%	3

Component 4 score:_____

Component 5: Sleep disturbance—questions 5b-5j

Questions 5b to 5j should be scored as follows:	
Not during past month	0
Less than once a week	1
Once or twice a week	2
Three or more times a week	3

Sum of 5b to 5j scores	Component 5 score
0	0
1-9	1
10-18	2
19-27	3

Component 5 score:_____

Component 6: Use of sleep medication—question 6

Response to Q6	Component 6 score
Not during past month	0
Less than once a week	1
Once or twice a week	2
Three or more times a week	3

Component 6 score:_____

Component 7: Daytime dysfunction—questions 7 and 8

Response to Q7	Component 7/Q7 subscore
Not during past month	0
Less than once a week	1
Once or twice a week	2
Three or more times a week	3

Response to Q8	Component 7/Q8 subscore
No problem at all	0
Only a very slight problem	1
Somewhat of a problem	2
A very big problem	3

Sum of Q7 and Q8 subscores	Component 7 score
0	0
1-2	1
3-4	2
5-6	3

Component 7 score:_____

Global PSQI Score: Sum of seven component scores:___________

Copyright notice: The Pittsburgh Sleep Quality Index (PSQI) is copyrighted by Daniel J. Buysse, M.D. Permission has been granted to reproduce the scale on this website for clinicians to use in their practice and for researchers to use in non-industry studies. For other uses of the scale, the owner of the copyright should be contacted.

Citation: Buysse, DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ: The Pittsburgh Sleep Quality Index (PSQI): A new instrument for psychiatric research and practice. Psychiatry Research 28:193-213, 1989

Appendix 7

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PROMISE Study

Stool Sample Collection

Your take home pack contains:

1. Two labelled screw-top scoop containers
2. Two labelled larger plastic containers
3. Disposable gloves
4. Kidney dishes
5. One zip-lock plastic bag
6. Two brown paper bags
7. 2 Ice-sheets
8. Chiller carrier bag

To collect the stool samples:

- Each container will be already labelled with your study ID. Please write the **DATE** and **TIME** of collection on all labels (two containers) with a ball-point pen.
- Place something in the toilet to catch the stool, such as the kidney dish provided, potty or an empty plastic food container, or spread clean newspaper over the rim of the toilet. You may line the kidney dish with toilet paper and flush the toilet paper away after you have collected the sample from the kidney dish.
- Make sure the stool doesn't touch the inside of the toilet.
- Use the scoop that is inside the lid of one of the small screw top containers to collect some of the stool, aim to fill around a third of the container (about the size of a walnut).
- Repeat this process with the second small screw top container and fill around a third of the container.
IT IS IMPORTANT TO PLEASE COLLECT 2 SEPARATE SAMPLES from the same stool by using both of the scoop containers we have provided.
- Screw on the lids of the two small containers containing the stool samples tightly.
- Fill each of the two larger containers with about 2cm of water and place the two smaller scoop containers inside the larger containers so that the small containers are surrounded by water (one small container inside one larger container). Screw on the lid of each of the larger containers tightly.
- Place each large container in a brown paper bag and place both containers into the ziplock bag.
- **PLACE IN THE FREEZER DIRECTLY OR AS SOON AS POSSIBLE.**
- At the same time as you place your samples in the freezer you can prepare your ice-sheets by soaking them in warm water and also placing them in the freezer.
- Deliver the two separate stool samples to us when you return to the Human Nutrition Research Unit on your second visit.
- **PLEASE TRANSPORT YOUR FROZEN STOOL SAMPLES TO US IN THE CHILLER BAG WITH THE ICE-SHEETS AROUND THE SAMPLES TO KEEP THE SAMPLES FROZEN. SAMPLES SHOULD NOT BE LEFT OUT OF THE FREEZER FOR MORE THAN AN HOUR.**