PROBIOTICS AND INTERMITTENT FASTING TO IMPROVE PREDIABETES IN OBESE PARTICIPANTS

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Abstract

New Zealand has the third highest rate of obesity within OECD countries with 8% of the adult population presenting type 2 diabetes and 25% living with pre-diabetes. Obesity and type 2 diabetes significantly increase the risk of cardiovascular disease and cancer and are critical modifiable risk factors associated with these conditions. In this study we determined whether relative loss of fat from visceral (compared to subcutaneous fat) depots can be manipulated by probiotics among participants with obesity and pre-diabetes who restricted caloric intake through intermittent fasting (IF). A randomized double-blind, two parallel arm study was performed in Auckland, New Zealand. Of the total 33 participants who were randomized, 26 completed the study, of whom 22 had magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) data at baseline and 12 weeks after starting treatment. All participants commenced 12-week of intermittent fasting (IF) as 2 days per week of caloric restriction to 600-650 kcal/day (IF5:2) and were randomized 1:1 to either daily probiotic (*Lacticaseibacillus rhamnosus HN001*) or matching placebo during this period. subcutaneous adipose tissue (SCAT), visceral adipose tissue (VAT), liver fat (LF), and pancreatic fat (PF), as quantified through MRI/MRS, were compared in probiotic versus placebo groups. Changes in fat depots were also compared with changes in haemoglobin A1c (HbA1c) and body mass index (BMI.) Overall, the average weight loss ranged from 94.2 to 89.5 kg and was mild-positively correlated with percentage of VAT to SAT ratio (%V:S) ($r = 0.215$, ($p = 0.392$). There was a correlation with %V:S and improvements in HbA1c ($r = -0.007$, $p = 0.973$). There was no difference in %V:S between probiotic versus placebo groups at 12 week. Intermittent fasting resulted in a modest weight loss of 5% which, when combined with probiotics, is not able to better target visceral fat loss compared to placebo. There was a correlation between %V:S fat reduction and improvement in HbA1c in overweight people with pre-diabetes. Therefore, intermittent fasting is beneficial in reducing weight and improve biomarkers in obese pre-diabetic participants, however, the beneficial effects of probiotics need to be further investigated for proper conclusion.

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List of abbreviations

ADA	American diabetes association
BAT	Brown adipose tissue
BCM	Body cell mass
BIA	Bioelectrical impedance analysis
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BP	Blood pressure
CDC	Center for disease control and prevention
CER	Continuous energy restriction
CV	Coefficient of variation
CRFs	Chronic renal failures
CVDs	Cardiovascular diseases
DPA	Dual photon absorptiometry
DPP	Diabetes prevention program
DXA	Dual-energy X-ray absorptiometry
EE	Energy expenditure
FFM	Fat-free mass
FPG	Fasting plasma glucose
GBP	Gastric bypass
GDM	Gestational diabetes
GFR	Glomerular filtration rate
GWAS	Genome wide-association studies
HbA1c	Hemoglobin A1c
HDL	High-density lipoprotein
HOMA-IR	Homeostatic model assessment of insulin resistance
IDEAL	Iterative decomposition with echo asymmetry and least

IF	Intermittent fasting
IGT	Impaired glucose tolerance
IL	Interleukin
IR	Insulin resistance
IRS	Insulin receptor substrate
IS	Insulin sensitivity
LBM	Lean body mass
LDL	Low-density lipoprotein
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MS	Metabolic syndrome
NCEP-ATP III	National cholesterol education program adult treatment panel III
NGT	Normal glucose tolerance
NIH	National institutes of health
NZ	New Zealand
OGTT	Oral Glucose Tolerance Test
OS	Operating system
PF	Pancreatic fat
PG	Plasma glucose
PIP	Probiotic in pregnancy
PPAR-r	Peroxisome proliferator-activated receptor-gamma
RCT	Randomised clinical trial
REE	Resting energy expenditure
ROIs	Region of interests
RQ	Respiratory quotient
SCAT	Subcutaneous adipose tissue
SF-12	12-Item Short-Form Health Survey
T2D	Type 2 diabetes

TBB	Total body bone
TBF	Total body fat
TC	Total cholesterol
TGs	Triglyceride
TNF	Tumour necrosis factor
VAT	Visceral adipose tissue
WHO	World health organisation
WHR	Waist to hip ratio

Attestation of Authorship

“I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly define in the acknowledgements), no material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning’’.

Dech Dokpuang

Dedications

This thesis is dedicated to my beloved parents, particularly my mother, who supported and motivated me to have a higher education, to my lovely sisters, Surat and Karaket, for their extreme encouragement for me to accomplish my study, to my supervisor Professor Jun Lu, my co-supervisor Associate Professor Rinki Murphy and all my teachers who made the way possible to reach this level of education. Finally to my father's and grandmothers' spirits, whom I both missed in their passing over my PhD period and had no chance to see them before they left me forever.

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Ethics Approval

Ethical Approval was received from New Zealand regional ethics committee (16/STH/107). This study was prospectively registered at ANZCTR (ACTRN12616001050448)

CHAPTER ONE

INTRODUCTION

Study background

It is estimated that approximately 71% of all global deaths are caused by non-communicable diseases (1), with Type 2 diabetes (T2D) being no exception, as it is one of the largest and fastest-growing non-communicable diseases in New Zealand (2). Coppell et al. (3) reported that 8% of the adult population have T2D, rising at a rate of 7% per annum, with 25% of them having pre-diabetes. Individuals are classified as having "pre-diabetes" when they have: (1) a fasting blood glucose between 100 and 125 mg/dL, (2) plasma glucose between 140 and 199 mg/dL after a 2 hour oral glucose tolerance test, or (3) haemoglobin A1c (HbA1c) between 5.7% and 6.4% (4). Lifestyle modifications, such as dietary and exercise changes in combination with weight loss purposes, are recognised as the first line of defense for combating these issues. Dietary counselling and increased physical activity have been studied as tools for preventing the occurrence of T2D, especially in pre-diabetic participants(5, 6).

The body mass index (BMI) measurement is widely utilised as an indicator of obesity, however, it is not the best indicator of this condition. Therefore, it is crucial to measure other indices for body composition, such as neck circumference measurements (which could reflect upper-body fat indirectly (7)), and waist to hip ratio (WHR) which is a common calculation used to predict the risk of T2D (8). These two types of data mentioned are utilised as a crude scale of fat distribution. Moreover, waist circumference (WC) has a direct relationship with abdominal content such as subcutaneous adipose tissue (SCAT) and visceral adipose tissue (VAT) (9). Waist circumference measurements are also considered to be more reliable markers when predicting metabolic syndromes (10).

Continuous energy restriction (CER) and intermittent fasting (IF) have long been studied as approaches for reducing weight and promoting better metabolic complications (11). However, due

to poor compliance of participants and high drop-out rates during study (12), IF has been conducted as an alternative to CER. In an IF intervention study, a participant was asked to limit their energy intakes to only 500-600 kcal per day, 2 days per week. For the remaining days of the week, dietary consultation was conducted to make sure that the participant would not consume any extra calories beyond baseline requirements. It is worth noting that the IF intervention study yields a comparable outcome with CER outcomes (13, 14).

Caloric restriction is the fundamental basis for weight loss. The early remission of diabetes following bariatric surgery is largely thought to be due to severe caloric restriction (15, 16). However, long term maintenance of restricted caloric intake without bariatric surgery is extremely difficult, often resulting in the failure of most cost-effective lifestyle programs (17, 18). Whilst long term, daily, very low calorie restriction is unsustainable, IF reduced intake may be more achievable with similar benefits in metabolic health, as with bariatric surgery (19). IF has been practiced by various religious groups for centuries and may be equally accessible across those of different socioeconomic and cultural backgrounds. It is believed that freedom in choosing the types of food to be consumed is important in long term adherence to nutritional advice, therefore, previous studies believe that a self-selected meal plan for intermittent fasting days is adaptable to current typical dietary patterns. Cultural food preferences and budget choice of participants, as well as freedom to choose the two fasting days in each week, seemed to be the preferable format method. Whether or not intermittent fasting, regardless of chosen method, is an effective technique in preventing the progression of pre-diabetes is currently uncertain.

Gut microbiota (the microorganisms colonizing our intestines) have emerged as a novel mediator of obesity and T2D through the modification of dietary energy extraction, hunger stimulation, inflammation, and glucose and lipid metabolism (20, 21). The most compelling evidence for a causal role of gut microbiota in obesity and metabolic disease comes from both animal and human experiments in which faecal transplants were able to alter recipient bodyweight and insulin sensitivity independent of energy intake (22, 23). Probiotics defined as "microorganisms able to confer defined health benefits to the host," work in complex and poorly understood ways to alter

macro and micronutrient availability from the gut (24), modify lipid and cholesterol metabolism (25) and exert anti-inflammatory responses (26), often in a microbe strain-specific fashion.

In light of this, the effects of probiotics in diabetes are strongly encouraging. The combination of *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* BB12, (10^{10} colony forming units each daily) was successful in reducing the incidence of gestational diabetes from 36% among women given placebo to 13% in those who received the probiotics combined with dietary advice (64% decrease) in a randomised controlled trial among 256 Finnish pregnant women (27). There was a 144 g reduction in birthweight and a further reduction in maternal adiposity 6 months postpartum with probiotic treatment (27). Other types of *Lactobacillus* probiotic supplementation have been demonstrated to improve insulin sensitivity (28), reduce body weight (29) outside of pregnancy, as well as greater levels of early weight loss after gastric bypass (GBP) surgery (30). *Bifidobacteria* concentrations have been associated with weight reduction (31). *Bifidobacterium* genera are healthy commensal bacteria, which are less common in the gut microbiome of obese mothers and those who gain excessive weight in pregnancy (32). In a double-blind, randomised, placebo-controlled, probiotic in pregnancy (PIP) study of *Lactobacillus rhamnosus* HN001 (HRC 11/318), 423 women with a personal or partner history of atopic disease were randomised to receive daily HN001 ($6x10^9$ cfu) or matching placebo. The incidence of gestational diabetes (GDM) was significantly lower in the HN001 group. This was most significant among high-risk women defined as obese, of older age, or with previous GDM.

Fat accumulation in the pancreas has been recognized as a consequence of obesity (33). Pancreatic steatosis in obese individuals has a strong link to pancreatitis (34), metabolic diseases and diabetes -- especially Type 2 diabetes (T2D) (35). The relationship between pancreatic fat (PF) and insulin resistance (IR) has been studied both in animal models and in human participants (36, 37), with PF being shown to have a strong association with BMI, IR and metabolic syndromes(MS) (35, 38). However, whether beta-cell dysfunction is a consequence of pancreatic fat accumulation has remained unclear. Van Raalte et al. (39) found that there was no lipid accumulation of intra-islet

cells in their 2010 study, with similar results shown in Van der Zijl et al.'s study in 2011 (40). On the other hand, Rasouli et al. (36) found that there is a negative association between beta-cell function and PF in normoglycaemic participants. Heni et al. (41) confirmed this association by conducting the experiments in pre-diabetic subjects. Lim et al. (42) found that T2D has a strong relationship with a smaller pancreatic volume and higher PF contents, when compared with healthy subjects. Harvie et al. (43) found that IF was superior to Caloric restriction (CR) in terms of insulin sensitivity and body fat reduction.

The pancreas is among one of the most difficult organs to study non-invasively as it lies deep in a retroperitoneal location, often with irregular boundaries and variable shape (44). The advancements of studying the links of PF with other metabolic syndromes has been halted by lacking precise PF quantification protocols and a consensus cut point. A recent review by Singh et al. (45) proposed that PF of 6.2% should be introduced as a cut-off point for further prospective studies. A conventional method of fat quantification involves freehand drawing around sample images to create regions of interests (ROIs) that lead to observer dependent tasks for fat quantification, but it is an extremely time-consuming task (15, 46, 47). The method is prone to vary across observers. In order to solve this issue Maggio et al. (48) utilized only 9 ROIs for each case as a representative of the entire pancreas. By placing ROIs in the head and tail of the organ, three slices were averaged for each participant for PF estimation. The authors showed quick and reliable results for PF that could reduce working hours for PF measurement. Al-Mrabeh et al. (49) developed a more reliable protocol for the MR-OPSY method. By applying three ROIs on the head, body, and tail of the pancreas, the fat contents could be calculated from these selections. The author also extended the use of this method to their previous studies (46, 47) in which the percentage of coefficient of variation (% CV) of PF measurements could be reduced dramatically after the final PF calculation. However, a longer intervention study to assess the reliability of this method has not yet been done.

Problem statement

The exact prevalence of diabetes in New Zealand is unclear because undiagnosed diabetes is expected to be very high; it is known that the prevalence of diabetes in New Zealand is increasing sharply. The prevalence of diabetes is 7% worldwide, while the prevalence of prediabetes is 25.5 % in NZ. There is a significant difference in diabetes prevalence between males and females in New Zealand as it is higher among males compared to females (8.3 % vs. 5.8 %) (50). According to Coppell et al. (3), the prevalence of undiagnosed diabetes is higher among Pacific people, and this incidence also varies among other ethnicities. The current population of New Zealand faces an alarmingly high risk of diabetes. A recent study showed that the working age population (35-44) are most at risk of diabetes (50). Despite the available information on diabetes treatment in New Zealand (51, 52), finding an appropriate diabetes management program is still unsuccessful. The occurrence of diabetes is not restricted to an exact population, and the prevalence is distributed disproportionately. The prevalence of diabetes is higher among low-income individuals (53). It has been predicted that the prevalence of diabetes will be increased by 70 % in 2025, particularly among children aged 5 and less (54). Diabetes as a cause of death is underreported. Based on the latest World Health Organisation (WHO) report, the eighth leading cause of death in both males and females (and fifth overall in females) is caused by diabetes. Diabetes, per se, can be the cause of micro and macrovascular diseases; T2DM leads to disability and premature death. Currently, more than 330 million people live with diabetes, and it caused more than 1.5 million deaths in 2012 (55). Although lifestyle changes will play a major role in attenuating the prevalence of diabetes, its prevalence is still predicted to increase to more than 330 million people (56). Along with total prevalence increase, it is estimated that the rate of death will soon increase to 3.7 million. This number includes 1.5 million diabetes-related deaths and another 2.2 million deaths due to comorbidities such as cardio vascular diseases (CVDs), chronic renal failures (CRFs) and tuberculosis related to hyperglycemia. Of note, hyperglycemia causes about 7% of diabetes-related mortalities among males aged between 20 and 70 and 8% within females at the same age (55). New Zealand is not an exception to these statistics.

It is estimated that up to 37% of patients with pre-diabetes who fail to manage or improve their condition, may see themselves develop diabetes within 4 years (57, 58). Studies on lifestyle changes over a long-term period have shown that the risk of this progression could be reduced from pre-diabetes to diabetes for an extended period of up to 10 years (58). Pre-diabetes is considered as a critical phase, because there is evidence that this condition is still reversible and could be considered as a potential approach to prevent diabetes (58, 59). Diabetes prevention programs (DPP) have shown that proper intervention in pre-diabetes could potentially reduce the development of T2D by up to 58% (60).

Continuous energy restriction (CER) has long been studied as an approach for reducing weight and improving metabolic complications (11). However, due to poor compliance and high drop-out rates in studies (12), intermittent fasting (IF) has been conducted as an alternative to CER. There is growing evidence linking gut dysbiosis with obesity and diabetes. Animal studies have suggested that probiotics can improve insulin sensitivity and beta-cell dysfunction by regulating the key signaling pathways (61, 62). Recent evidence from a New Zealand study showed that the probiotic *Lacticaseibacillus rhamnosus* HN001 (previously known as *Lactobacillus rhamnosus* HN001) can result in improved (decreased) gestational diabetes prevalence despite no changes in body weight (63). However, no study has examined the effect of probiotics in combination with intermittent fasting in which body fat measurements along with other biomarkers were calculated.

Hypothesis

Intermittent fasting (5:2) with or without probiotic supplementation contributes to improving glycemia and weight among overweight New Zealand adults with pre-diabetes

Overall aim

To assess the efficacy of intermittent fasting (5:2) with or without probiotic supplementation for improving glycemia and weight among overweight New Zealand adults with pre-diabetes

Specific objectives

To determine if the intermittent fasting intervention and/or use of probiotics achieves superior glycemic reduction as measured by HbA1c at 1 year

To assess impact of intermittent fasting intervention and/or probiotics on body weight at 1 year

To assess the relationship between body weight loss and reduction in liver fat and pancreatic fat with improvement in glycemia

To access an uptake and acceptability of the interventions

To identify social and biological markers for predicting the effectiveness of these interventions

To assess impact of the interventions on appetite, appetite regulating hormones, inflammation, metabolomic profiles, gut microbiota, RMR, and body composition

CHAPTER TWO

LITERATURE REVIEW

Worldwide Prevalence of Diabetes and Prediabetes

Diabetes has no cure and consists of four common types: Type 1, Type 2, gestational diabetes, and "additional type." About 90-95 % of all kinds of diabetes are T2DM. In 2005, diabetes was identified as the fifth leading cause of death in the world. Every day more than 4000 people are diagnosed with diabetes, and more than 650 people die from diabetes. It has been shown that the rate of death has increased by 45% from 1987. In the US more than $132 billion is spent on diabetes and its morbidities each year, being responsible for 11% of public health costs alone(1). It has been predicted that the prevalence of diabetes will be increased to more than 330 million people by 2030 (1). Nevertheless, good glycaemic control by either changing lifestyle or using another effective treatment may decrease this prediction significantly. For example, in a study from a randomised clinical trial called the Diabetes Prevention Program (DPP), it has been shown that the incidence of T2D decreased by 58% through lifestyle changes and 31% through use of pharmacologic agents (metformin) (2). According to the latest International Diabetes Federation (IDF) report, more than 415 million people currently live with T2DM, and it has been predicted this number will reach 642 million by 2040 (3). Diabetes is an economical, global burden. Most people who are affected by diabetes are in the working ages (40-59 years old). The Western Pacific, where New Zealand is located, has the highest prevalence of diabetes. According to the IDF report, every six seconds a person dies in the world due to diabetes. As recently as 2013, about $548 billion was spent on this catastrophic disease, with the amount of money projected to reach $627 billion by 2035. In the Western Pacific, 138 million people suffer from T2D while 54 % of these people remain undiagnosed. Almost 16 % of deaths in the Western Pacific are due to diabetes and its complications. Unfortunately, half of those who have died from diabetes were aged less than 40 years old (4). It is therefore important to find inexpensive and user-friendly interventions which can reduce the higher expenditures that are predicted for diabetes treatments. Figure 2.1 shows the global outbreak of

T2D with a projection of 113 million diabetic patients by 2030 in the Western Pacific region (Adapted from (5)).

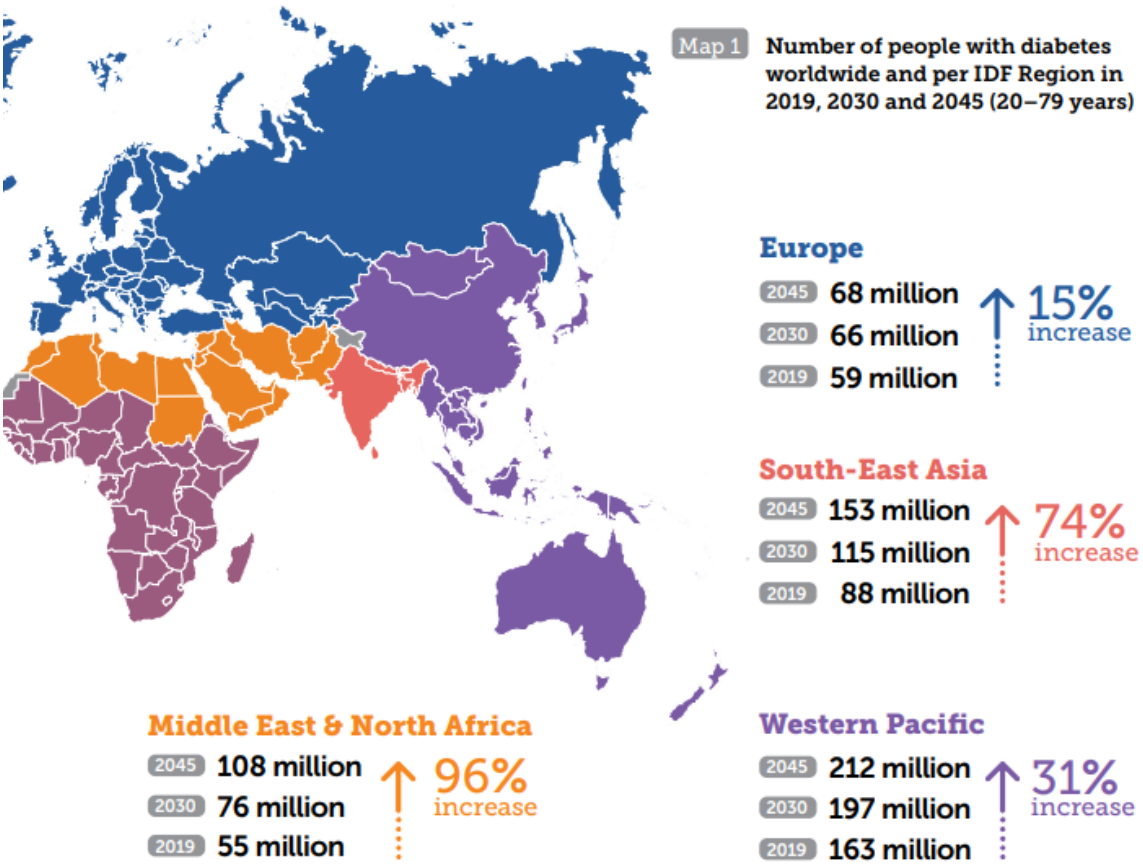

Figure 2.1 worldwide prevalence of diabetes

National Prevalence of Diabetes and prediabetes

The exact prevalence of diabetes in New Zealand is unclear: The prevalence of undiagnosed diabetes is expected to be very high, and it is also greatly increasing. Worldwide, the prevalence of diabetes is estimated to be 7 %, while the prevalence of prediabetes is 25.5 % in NZ. There is a significant difference in diabetes prevalence between males and females in New Zealand, as it is higher among males compared to females (8.3 % vs. 5.8 %) (6). According to Coppell et al. (6), the prevalence of undiagnosed diabetes is higher among Pacific people, and this also varies among other ethnicities. The current population of New Zealand faces an alarmingly high risk of diabetes.

A recent study showed that the working age population (35-44) are most at risk of diabetes (6). Despite the available information on diabetes treatment in New Zealand (7, 8), finding an appropriate diabetes management program is still unsuccessful. Changing one's lifestyle is highlighted more than other interventions (9) to prevent or at least control diabetes. It is important for public health sectors and researchers to make an appropriate plan for people who are more at risk of diabetes and its complications, namely people who are obese or overweight (10). Obesity plays a major role in the development of diabetes in New Zealand. Although in comparison with the US, the incidence of extreme obesity is significantly lower among New Zealand people (4 % vs. 2.7 %, respectively), there is a significant difference in ethnicities across which the data is reported in New Zealand. It has been shown that Europeans have a lower prevalence of diabetes (0.8 %), with moderate prevalence in Maori with 5.1 %, and a higher prevalence within Pacific Islander ethnicities, 10.9 % (11). Diabetes is a "silent killer", and the study of diabetes remission is a major issue for New Zealanders. In 2009, 869 people died due to diabetes in New Zealand (12).

Pre-diabetes and diabetes diagnosis criteria

Pre-diabetes, typically defined as glycaemic parameters above normal levels but below diabetes thresholds, is a metabolic state between normal blood glucose and diabetes, including impaired fasting glucose, impaired glucose tolerance, and impaired fasting glucose combined with impaired glucose tolerance (13). The diagnosis of pre-diabetes is shown in Table 2.1. Socio-economic development and the change of human diet structure are indicators of the prevalence of diabetes and pre-diabetes. Both states are increasing rapidly. The number of diabetic patients worldwide is projected to rise from 382 million in 2013 to 592 million in 2035 (14). It is estimated that 470 million people worldwide will suffer from prediabetes by 2030, and 5 % to 10 % of pre-diabetic patients will develop diabetes every year (13). Diabetes causes major health problems as well as a large economic burden. The direct annual cost of diabetes worldwide exceeds US$827 million (15). Moreover, diabetics lose a great deal of labor value due to reduced productivity and production

time. Consequently, timely and effective preventive measures in pre-diabetes are a reasonable way to impact the diabetes epidemic and lessen the healthcare cost.

Table 1.1 criteria for diagnosis of prediabetes with 100 g or 75 g glucose load

	mg/dl	mmol/l
100-g glucose load		
Fasting	95	5.3
1-h	180	10.0
2-h	155	8.6
3-h	140	7.8
75-g glucose load		
Fasting	95	5.3
1-h	180	10.0
2-h	155	8.6

American Diabetes Association Diabetes Care 2010;33:S62-S69

To be diagnosed as diabetic, one's blood glucose level needs to be equal to or above a certain value. According to the American Diabetes Association (ADA), there are four methods for the diagnosis of diabetes, and the same methods are used for the screening of pre-diabetes in patients (16). The methods are:

(1) Fasting plasma glucose test (FPG): where fasting refers to the absence of food and drink intake, apart from water, for at least 8 hours before the test;
(2) Oral glucose tolerance test (OGTT): where a patient consumes a glucose syrup solution containing 75 g of glucose after which a blood test is carried out to determine 2-hour plasma glucose (2-h-PG);
(3) HbA1c (Glycated hemoglobin or hemoglobin bonded to glucose) levels via a laboratory test; and
(4) Random PG of more than or equal to 200 mg/dL or 11.1 mmol/L in patients that displayed symptoms of hyperglycemia or hyperglycemic crisis.

Many studies, as reported above, have been done on diabetic patients, however, little is known about the pre-diabetic condition. Pre-diabetes is a term used to describe the buffer period before the onset of T2D, where the blood sugar level is higher than normal but lower than the diagnostic criteria of T2D. Impaired β-cell function and increased insulin resistance are two pathological pathways that lead to pre-diabetes, and subsequently, diabetes. The onset of increased insulin resistance starts years before diabetes and even pre-diabetes (13). More specifically, insulin resistance in skeletal muscle tissues can be regarded as the initiating factor that is present decades before impaired β-cell function (17). Additionally, in a study conducted by Cerasi et. al. (18), it was observed that there was a decrease in glucose-induced insulin release of the pancreatic β-cells in diabetic and pre-diabetic patients. The dose–response curve for glucose-induced insulin release shifted to the right and further right for pre-diabetic and diabetic patients, respectively, as compared to normal individuals (18). Moreover, it was also reported that there was a significant increase in β-cell function 3 to 4 years before diabetes diagnosis, followed by a steep decrease in function (13). Combining the continued increase in insulin resistance with a decrease in β-cell function, glucose levels in the blood become unregulated and pre-diabetes then evolves into full-blown diabetes.

Diabetes Prediabetes and Ethnicity

It is clear that different ethnicities have a different proportion of diabetes. For example, people from Native American, Polynesian or Micronesian, Asian-Indian, Hispanic, or African-American descent are at higher risk of diabetes in comparison to people of European ancestry (19). In New Zealand, the prevalence of diabetes is greater among Pacific people, with the lowest prevalence among the European population (20). The largest proportion of new or known diagnosed diabetes belongs to Pacific men and women aged 45-64. Furthermore, in regard to impaired glucose tolerance (IGT), Pasifika and Māori in the working ages < 45 have the highest prevalence of IGT (7). Pacific people constitute almost 8 % of the New Zealand population (21). Diabetes diagnosis is very simple, yet more than half of all diabetes cases remain undiagnosed, especially among Pacific peoples (20, 22). Being Maori or Pasifika plays an important role in the increased risk of diabetes

in New Zealand (7). It is now clear that the prevalence of diabetes is very high within the Maori population as well (8, 23, 24). Ethnicity has also been found to influence the complications of diabetes, for example, Maori renal complications and deaths from renal diseases are significantly higher than those of European descent (25). Accordingly, the highest risk populations should be considered as a priority to try any successful treatment for diabetes.

Obesity

Obesity is a significant issue in public health that is increasing rapidly, particularly in industrialized nations (26). The most widely accepted classification of obesity is based on the BMI. In this classification, obesity has three grades: Grade 1 or overweight with a BMI of 25-29.9 kg/m^2, Grade 2 or obesity with a BMI of 30-39.9 kg/m^2 and Grade 3 or morbid obesity (severe obesity) with a BMI ≥40 kg/m^2 (2). Obesity is also categorized according to body fat percentage. That is, percentage of body fat >25%, with 21-25% being borderline and percentage of body fat >33%, with 31-33% being borderline for men and women respectively (27). There are several kinds of measurements used to evaluate obesity, such as BMI, waist circumference, waist/hip ratio calculation, Dual-energy X-ray absorptiometry(DXA), and Bioelectrical impedance analysis(BIA). The link between diabetes and obesity goes back many years when Elliott P. Joslin introduced obesity as the risk factor of T2D for the first time in 1921, mentioning that the rate of diabetes is higher among obese people (28). Obesity is the sixth greatest health risk regarding the leading burden of disease globally (29). It was shown that a mere weight loss of 1 kg resulted in a decrease in the risk of diabetes by 20 %. Additionally, it has been documented that 5 % body weight loss, or a 5 kg weight loss, resulted in decreasing the incidence of diabetes by 58 % (30). In another study, researchers showed that the risk of diabetes increases by four-fold in people who are aged 20 to 44 years old (31). In a cohort study of more than 50,000 people, it was reported that the risk of diabetes is strongly related to BMI. The higher an individual's BMI, the greater risk of diabetes. Therefore, BMI is considered to be the dominant risk factor for T2D (32). Although the exact mechanism of obesity in the development of IR and T2D is unclear, adipose tissue likely plays a vital role through acting on fat, liver or skeletal muscles to impair insulin activity. It has been shown that obesity is associated with

hyperinsulinemia and increased levels of insulin, and it may stimulate IR via downregulation of insulin receptors. The potential candidate factors to contribute to IR are mostly adipocyte-based substances such as resistin and adiponectin, and inflammatory factors such as interleukin-6. Furthermore, free fatty acids are capable of inhibiting insulin activities (33). Despite the increasing pace of gains in health-related knowledge, there is still no accurate treatment for obesity. The role of obesity research must be to find an accurate and precise solution to remit obesity. Obesity management at first is usually followed by an intensive and a comprehensive lifestyle change such as diet, pharmacotherapy and later, for those who have continually unsuccessful treatments, bariatric surgery is chosen as a final intervention (34-37).

Diabetes and pre-diabetes Risk Factors

Over the last half-century, lifestyle changes have led to a significant increase in the outbreak of diabetes all around the world. Physical inactivity, increased food consumption, and aging are the most important risk factors for diabetes. Nevertheless, genetic predisposition and socioeconomic status are not to be ignored. This is the most readily obvious among Maori and Pacific populations. The family history of diabetes has also been introduced as an independent risk factor for diabetes (38). T2D is no longer accepted as only a disease of adult people; as diabetes prevalence increases, the age of diabetes onset is also reducing. In New Zealand, one out of six people over 60 years old has diabetes. Additionally, lower income households have a negative correlation with the development of diabetes (39). Uncontrolled glycaemia is another important issue identified as a risk factor for diabetes. Control of glycaemia is critical to the reduction of future hazards. It has been shown that even a single event of reducing blood glucose levels to a normal range reduces the future of diabetes risk factors significantly (40). Perreault et al. (40) reported that each time glucose is reduced within a diabetic group it results in decreasing the risk of diabetes. For instance, a single one-time, second time and third-time reduction of glucose are equal to 56 %, 61 % and 67 % reduction of diabetes complications, respectively. Any effort to reduce blood glucose to normal levels may increase life expectancy and healthy living for diabetic patients. Perreault's study also showed that age plays a major role in diabetes risk factors. Young age participants had a higher risk of future

diabetes (age < 45 years). Notably, taking some medicines such as large doses of exogenous steroids may cause diabetes (41). Other common risk factors for diabetes include hypertension, hyperlipidaemia and gestational diabetes (3). In New Zealand, as with other countries, obesity, gaining weight, no exercise, and low consumption of fibre-containing foods are the most important risk factors for T2D (42-45). The impact of weight loss to improve diabetes is accepted universally (3, 46). Due to the significant role of obesity in the development of diabetes, it will be explained in more detail in the next section. Diabetes is a cocktail of several risk factors. Therefore, the exact underlying mechanism of diabetes is still questionable. Research efforts, therefore, should be focused on finding the best way to control and prevent diabetes. Despite the enormous consensus about the crucial role of diabetes prevention, there is still no effective approach to the prevention of the disease (47).

Obesity

T2D occurs due to various factors that cause insulin resistance and β-cell dysfunction. Various cross-sectional and prospective studies have unanimously confirmed that obesity is one of the environmental factors that has a correlation with T2D. A total of 50 % of people with T2D are obese (BMI > 30 kg/m2), whilst a total of 90 % of diabetic patients are overweight (BMI > 25 kg/m2). Therefore, even just a moderate weight loss can have an immense impact in controlling diabetes (48, 49). Due to obesity, the levels of adipocytes, cytokines (interleukin-1 (IL-1) and interleukin-6 (IL-6)), and tumor necrosis factor alpha (TNFα)) increases in the body. The increased amount of these components triggers a signaling pathway which represents an inflammatory action of the adipose tissue (50). This chronic low-grade inflammatory action might promote insulin resistance in cells (51). In a study conducted by Barbarroja et al. (50), it was found that the level of mRNA expression of IL-1β and IL-6 among insulin resistant obese people was high in comparison to non-insulin resistant obese patients. Furthermore, a study conducted on the health of nurses, as reported by Wild and Byrne (52), found that women whose BMI was greater than 35 had a 49-times greater chance of developing T2D compared to those whose BMI was less than 22. Studies on men have shown similar results, with Wild and Byrne (52) reporting that men with a BMI greater than or

equal to 35, had a 42-times higher chance of developing the disease than men with a BMI less than 23. This study was conducted on a cohort of men from the United States of America. For Asians (mostly Indians), the onset of higher risk of diabetes starts at an even lower BMI value (15–20). Recent studies have shown that larger waist circumference could be a better indication of developing T2D than BMI. Several studies in China, the U.S., and Finland state that the risk of developing T2D can be lowered through decent weight loss (52).

Sedentary Lifestyle

Through various studies, a sedentary lifestyle has been proven to be one of the major causes of developing T2D. Watching television is one of the more popular sedentary activities, and it is also much worse than other sedentary activities, such as playing board games, sewing, reading, writing, or driving a car. This is because the metabolic rate while watching television is the lowest among the mentioned sedentary activities (53). A meta-analysis was conducted with 10 studies and consisted of 505,045 participants in total. In this analysis, it was found that people with longer TV time had a 112 % higher pool for T2D compared to the people with shorter TV time (54). The explanation for this can be tied to an individual's BMI, as people who tend to spend more time watching TV are less physically active, and thereby increase their BMI. The physiological reasoning is that during acute contraction of active muscles, there is an immediate uptake of plasma glucose, and when someone is physically inactive, this plasma glucose uptake does not happen often enough. The decrease in insulin sensitivity is another reason. A study was conducted by Balkau et al. (55) with 801 apparently healthy volunteers, to find a relation between sedentary time and insulin sensitivity. In the study, sedentary time was measured with accelerometry, and insulin sensitivity was measured with a hyperinsulinemic-euglycemic clamp. The study concluded that there was an inverse relationship between sedentary time and insulin sensitivity. The difference between the most and least physically active hours was only 4 hours, however, the insulin sensitivity ranged around 40 % (55). Therefore, by replacing just 30 min of sedentary lifestyle with moderate to vigorous physical activities, insulin sensitivity is expected to improve by 15 % (56). Moreover, since a sedentary lifestyle is strongly correlated with weight gain or obesity, the

pathophysiological paths of obesity causing diabetes may also be applicable to a sedentary lifestyle.

Ageing

A considerable number of studies have found that the likelihood of T2D increases with the increase in age. According to the National Diabetes Statistics Report of the United States of America, about 4.0 % of people had diabetes in the age group 18–44 years. This number increased to 17.0 % in the age group 45–64 years, and the percentage further rose to 25.2 % for the age group ≥ 65 years (57). Similar results were found in a survey conducted in England, where according to the Health Survey for England (2006), the highest prevalence of diabetes existed in the age group 65–74 years. A total of 15.7 % of the surveyed men in that age group had diabetes, whereas, in the same age group, 10.4 % of women had diabetes (58). In another study conducted by Suastika et al. (59) on a cohort in Bali, it was found that the prevalence of T2D was more than 2-fold in the older generation compared to younger people (59). A study conducted on elderly Chinese in 2000 in Taiwan found that 16.9 % of them had T2D. After 5 years, a follow-up survey was done on the same group of people and it was found that the prevalence of diabetes rose to 23.7 % (60). The most likely pathophysiological reason behind this is that the human body gets less sensitive to insulin as it ages, and similarly, β-cells are altered or show insufficiency in insulin production with aging (61).

Sex and Gender

According to a report published by the International Diabetes Federation (IDF) in 2017, the total number of adults (20–79 years) diagnosed for T2D was 425 million, which is 8.3 % of the total world population. The distribution was not equal between men and women in the findings as 17.1 million more men were diagnosed with diabetes than women (221.0 million were men and 203.9 million were women). The total number of diabetic adults is expected to rise to as high as 629 million by the year 2045, which is 48 % more than in 2017 (62). The imbalance in the prevalence of diabetes based on gender is yet to be understood, as more men are diagnosed with T2D, but women have more cases of obesity -- one of the leading causes of T2D. The probability of diabetes according to

sex and gender arises from various biological and environmental factors. In endocrinology, the most prominent effect of sexual dimorphisms is expressed through T2D. This might be due to the difference in sex chromosomes, sex-specific gene expression of autosomes, sex hormones and their effects on organ systems. Anatomically, men and women have different body fat distribution and brown adipose tissue (BAT). Furthermore, the healthy range of BMI value of men and women is also different, and the onset of risk of diabetes starts at a lower BMI value for men compared to women (63). Conversely, women have more obesity induced diabetes compared to men. In terms of fat distribution, men have more trunk and visceral fat (VAT), and liver fat, in comparison to women of the same BMI and age (64, 65). Moreover, men have a higher amount of VAT for any amount of total fat (66). Women have more deposition in leg fat tissue (67). This difference in fat distribution might be one of the underlying reasons for the difference in diabetes prevalence among the genders. Recent studies have found that the different level and activity of brown adipose tissue (BAT) in the genders may play a role as well (68). There is a negative relation between the activity of BAT and diabetes risk. Increased activity of BAT reverses obesity, increases adiponectin and reduces insulin resistance (69). Besides all of the aforementioned reasons, the difference in diabetes pattern among the genders may also be due to different exposures to environmental factors, such as: nutrition, healthcare facilities for prevention or treatment of diseases, lifestyle, socioeconomic status, psychosocial stress, sleep deprivation, work stress and many more (70-72). The extent of the effect of the factors is different for males and females.

Hypertension

Cases of diabetes and hypertension overlap significantly, and therefore, it is very difficult to understand if diabetes causes hypertension, or if hypertension causes diabetes. Recently, the American Heart Association regarded diabetes to be a risk, rather than a risk factor, of coronary heart diseases after determining that the risk of having myocardial infarction in diabetic individuals is equal to that of patients who had a history of previous myocardial infarction (73). Nonetheless, Cheung and Li have suggested that both diseases might carry the same or similar etiology and disease mechanisms (74). In the Hong Kong Cardiovascular Risk Factor Prevalence Study, Cheung

found that only 42 % of diabetic patients had normal blood pressure and 56 % of the hypertension patients had normal blood glucose level (75). Cheung and Li have concluded that both diabetes and hypertension are the results of a metabolic syndrome which is caused by obesity (74), and therefore, that obesity is a risk factor for both diseases

Smoking

Several long-term studies have concluded that people who are chronic smokers have a higher risk of developing T2D as compared to nonsmokers (76-79). In one study, it was found that people having 20 cigarettes a day had a 61 % higher risk of developing T2D, whereas, people having less than 20 cigarettes a day had only 29 % higher risk of developing T2D (77). The difference is evident and has been backed by various studies including one which showed that a higher dose of insulin was needed to achieve the same metabolic control in smokers as compared to nonsmokers. This risk arises due to insulin insensitivity resulting from nicotine, one of the active chemicals in cigarettes (80). By extension, it is not only smoking, but also nicotine patches, that have been found to decrease the effect of insulin (81), further confirming that nicotine is an active compound that contributes to diabetes. Smoking is found to severely aggravate glucose tolerance and the insulin sensitivity index (82). Bergman et al. (83) found that smokers have reduced expression of peroxisome proliferator-activated receptor-gamma (PPAR-γ), a transcription factor that promotes insulin sensitivity, as well as increased Serine 636 phosphorylation of insulin receptor substrate IRS-1, which results in decreased insulin signaling. Among people with a normal BMI, studies have found that smokers have higher abdominal obesity than nonsmokers, which is a key risk factor for diabetes (77, 84). Yun et al. (85) conducted a study in which they found that people smoking more than 20 cigarettes had a 1.93 abdominal obesity ratio compared to people who had never smoked. Smoking is linked to causing detrimental changes in body composition which might lead to the development of diabetes (86). Yoshikawa et al. (87) found that there are nicotine receptors on the pancreatic insulin-producing β-cells. Evidence from the same research indicates that not only does chronic exposure to nicotine increase the risk of T2D, but also that acute exposure causes a reduction in insulin sensitivity (87). Results from a study conducted by Bruin et al. (88) have also

shown that nicotine could cause pancreatic β-cell apoptosis and a reduction in pancreatic β-cell mass.

Studies conducted specifically on Asians have found the positive correlation between smoking and diabetes, with such trends being reported in China, Taiwan and Korea. The majority of Asian men (50 % to 60 %) are smokers (89-91). China is both the largest producer and largest consumer of cigarettes in the world, followed by another Asian country, India. More than 33 % of cigarettes are consumed by people in China alone (92, 93). In a study conducted on 513 Japanese men, there was a positive correlation between smoking and higher waist-to-hip ratio (visceral adipose: subcutaneous adipose) (86), therefore, the impact of smoking on diabetes is likely to be intense among the Asian nations.

Alcohol

Alcohol is another risk factor for T2D when consumed above a certain threshold value, however, when consumed below the threshold value, it actually reduces the risk of T2D. The latest meta-analysis to find the relation between alcohol consumption and T2D was carried out by Knott et al (94). The meta-analysis reviewed 38 studies consisting of 1,082,639 male subjects and 819,966 female subjects. When alcohol was consumed at any amount below 63 g/day, there was a reduction in the risk of T2D, and that when alcohol consumption was 10–14 g/day, the reduction was highest. In contrast, when alcohol consumption increases above 63 g/day there is a positive correlation with the risk of T2D development (94).

Evidence for Genetic Risk Factors

Genetics is another major risk factor for T2D. Various studies have found that people of certain ethnic groups have higher chances of developing T2D than others. For example, Pima Indians living in Western countries have twice the risk of T2D than native Europeans. It was found that people whose parents have T2D have 6 times more risk of developing T2D, as compared to the control group. Therefore, people with parents who have T2D, have a 40 % higher chance of developing the same condition (95). Over the past 35 years, numerous studies have been conducted

to find if genetics is a reasonable risk factor of T2D. Park (6) found that family-based linkage analysis, candidate gene approach and genome wide-association studies (GWAS) were the three approaches used to find if genetics is responsible. In total, more than 40 genes have been identified as being responsible for T2D.

Progression of prediabetes to diabetes

It has been estimated that the number of pre-diabetic cases will increase to more than 470 million people worldwide (96). The Centers for Disease Control and Prevention (CDC) reported that in 2015, almost half (48.3 %) of the adult population aged 65 and above had pre-diabetic conditions, and that approximately 84.1 million people in the U.S. were already pre-diabetic. In accordance with the higher prevalence of diabetes in South Asian ethnicities, the Asian population showed a higher prevalence in pre-diabetes than the Western population (97). Studies have shown that non-East Asian countries such as Saudi Arabia (6.8 %), India (6.3 %) and South Latin America (17.8 %) have a pre-diabetes prevalence 2 to 5-fold lower than East Asian countries such as China (35.7 %). A total of 37 % of pre-diabetic patients who leave their condition untreated may see themselves develop diabetes within 4 years (98-102). However, if lifestyle changes were adopted, long-term studies have shown that the risk of this progression — from pre-diabetes to diabetes — can be lowered for an extended period of 10 years (99). Pre-diabetes is seen as the critical phase, because studies have shown that at this stage, the condition is reversible and could serve as a potential route to combat diabetes (99, 102). Thus, in this paper, we conduct a general review which includes pre-diabetic conditions, by pooling together publications that discuss diabetes from different perspectives, in the hope that with a better understanding of this disease we can provide invaluable insight as to how we can combat diabetes around the world.

Randomized Clinical Trial

A Randomized Clinical Trial (RCT) is defined as a prospective intervention study in human subjects to evaluate experimental drugs, a new therapy, medical instruments, or clinical methods. Research design in clinical trials plays a vital role in biomedical research. Clinical trials are divided

into two general groups: uncontrolled and controlled clinical trials. A clinical trial that does not involve a comparison between case and control group regarding a specific treatment or intervention, is called an uncontrolled clinical trial. On the other hand, controlled clinical trials involve a group of control subjects to compare with the case group. Randomized, double-blind (which means neither patients nor researchers know the identity of the intervention or treatment) studies have the highest validity and accuracy (103). If a clinical trial is well-designed and appropriately conducted it is a powerful way to make a conclusion on a given intervention. Whenever subjects meet the criteria, they enter into the study on different calendar days. All clinical trials have a study protocol which generally includes information about the background of the disease, rationale for the study, which methods will evaluate an intervention's efficacy and safety, and statistical design along with methodology ("statistical consideration"). In this design, the potential risks and advantages of the intervention, the size of sample, should be described. Furthermore, all clinical trials have a general objective and several specific targets; all objectives must be practicable and clinically significant. Bias is a systematic error in any clinical trial, and one of the best methods to reduce bias is to conduct a blind clinical trial. Another method is randomization which effectively monitors patients within different interventions. In an RCT, confounding effects can be reduced significantly (103). Randomization can be simply done by a computer. Therefore, using an RCT can be a powerful tool when investigating a new treatment or intervention for chronic diseases such as T2DM and obesity.

Prevention of diabetes from pre-diabetes by probiotics

Early intervention in pre-diabetes can reduce the risk of developing T2D by 58 % (104). Effective interventions include lifestyle changes and medications (105-107). However, in clinical practice, the adherence to lifestyle changes in pre-diabetic populations is low, and the compliance of using hypoglycemic drugs is relatively poor. Thus, to find a non-hypoglycemic agent is of great significance in preventing the conversion of pre-diabetes to diabetes. Recent studies indicate that alterations in gut microbiota play a major role in the pathogenesis of diabetes (108-110). It is

reported that when intestinal dysbacteriosis occurs, the production of lipopolysaccharide increases, release of pro-inflammatory cytokines increases, and a series of nonspecific inflammatory responses occur in the body, which interferes with insulin signal transduction, induce insulin resistance and lead to hyperinsulinemia (111), as well as trigger chronic low-level inflammatory responses of islet cells and metabolic endotoxemia, ultimately leading to destruction and apoptosis of islet β cells (112, 113). Microecological preparations, including probiotics, prebiotics, and synbiotics, have beneficial health effects on the host when administered in sufficient amounts. Studies suggest that certain microecological preparations can exert anti-diabetic effects (114, 115), improving glycemia, insulin sensitivity and inflammatory markers in subjects with T2D (116-119). Several human studies have also demonstrated the beneficial effects of microecological preparation supplementation in pre-diabetes (120, 121).

The pathophysiology of pre-diabetes

In healthy people blood glucose is strictly regulated. Fasting glucose is maintained between 3.9 and 5.6 mmol/L (122) and the post-meal increases rarely exceed 3 mmol/L(123). During the development of T2D, the homeostasis of fasting and postload glucose becomes abnormal (124).

As evidenced by studies with repeat measures of glucose levels, insulin sensitivity and insulin secretion, the development of diabetes from normal glucose tolerance (NGT) is a continuous process (125-128). Recently we described trajectories of fasting and postload glucose in addition to trajectories of homeostatic model assessment for insulin resistance (HOMA-IR) insulin sensitivity(IS) and insulin secretion (β-cell function) preceding the development of T2D in the British Whitehall II study (126). In people who developed diabetes, increased glucose values were observed at the beginning of the follow-up, 13 years before diagnosis, although glucose values seemed to be tightly regulated within the normal range until 2–6 years before diagnosis when an abrupt increase was found. This pattern of glycaemic changes was confirmed by other sources (125, 127, 128).

Weir (129) coined a multistage model of diabetes development that corresponds to the aforementioned findings. The first stage of diabetes development is a long compensatory period when insulin resistance is present and accompanied by increased rates of insulin secretion and an increased β-cell mass (124). The second stage is the stable adaptation period when β-cells are no longer fully compensating for increased insulin resistance; thus fasting and/or postload glucose values are not completely maintained. This period is likely to start when fasting and postload glucose levels are within the normal range (124, 125, 129) and is usually accompanied by a decrease in acute insulin secretion that is present at FPG levels around 5.6 mmol/L (124). Much of the first and second stages therefore occur before the pre-diabetic phase is achieved.

During the unstable early decompensation period, the third stage of diabetes development, the β-cells become unable to compensate for a given insulin resistance and consequently glucose levels start to increase rapidly (124, 129) as seen in Whitehall II and other longitudinal studies (125, 128). This period probably extends from pre-diabetes to manifest diabetes. The subsequent two stages of diabetes development (stable decompensation and severe decompensation) relate to manifest diabetes and thus are beyond the scope of this review (129).

Progression from pre-diabetes to diabetes

A range of 5–10 % of people with pre-diabetes become diabetic annually, although conversion rate varies by population characteristics and the definition of pred-iabetes (130, 131). In a meta-analysis of prospective studies published up to 2004, annualised incidence rates of diabetes for isolated IGT (4 – 6 %) and isolated IFG (6 – 9 %) were lower than those for IFG and IGT combined (15 – 19 %) (132). More recent major studies have similar progression estimates: the annualised incidence was 11 % in the Diabetes Prevention Program (DPP) Outcomes Study (133), 6 % among participants with IFG in the US Multi-Ethnic Study of Atherosclerosis (MESA) (134), 9 % among participants with IFG, and 7 % among those with an A1c of 5.7 – 6.4 % in a Japanese population-based study (135).

Studies suggest that the risk of diabetes development on the basis of FPG and 2 hour postload glucose is broadly similar to that posed by A1c (132, 136).

According to an ADA expert panel, up to 70 % of individuals with pre-diabetes will eventually develop diabetes. In a Chinese diabetes prevention trial, the 20-year cumulative incidence of diabetes was even higher (> 90 %) among controls with an IGT defined with repeated OGTTs (137). For comparison, women with gestational diabetes have been suggested to have a 20 % – 60 % risk of developing diabetes 5 to 10 years after pregnancy (138-140). This large heterogeneity in the estimates is probably due to the variation in the criteria used to define gestational diabetes and T2D in these studies. In a recent meta-analysis of 20 studies, 13 % of mothers with gestational diabetes developed diabetes after pregnancy compared to 1 % of mothers without gestational diabetes (141).

Biomarker Study

Biomarkers, also known as signature molecules and molecular markers, are "biological molecules that show health and disease states", and are currently used in basic and clinical research (142). Their role as essential endpoints in clinical trials is accepted universally. Some types of biomarkers are specific, meaning they have been well characterised and consequently are confirmed to predict given clinical outcomes across a diversity of treatments and populations. Nevertheless, the validity of a biomarker must be evaluated and re-evaluated (143). There are a couple of different biomarker definitions by WHO (144, 145), both overlap a concept: namely a link between biomarker and predicting the incidence of the outcome of a disease, and reproducibly at the same time for different populations. It is, however, important to know that biomarkers are always "provisional." Risk factors sometimes increase the chance of getting a disease. The most important effect of biomarkers is to detect a risk factor before appearing in the clinical pattern of a given disease. Biomarkers should be confirmed, at least, by two independent populations (142). For example, HbA1c is a well-known biomarker to evaluate glycaemic control for diabetic patients who undergo a given intervention (146). Taken together, biomarkers play a crucial role to pave a way to improve drug

discovery and biomedical research. Understanding a relationship between quantifiable biological substances and clinical outcomes is essential to expand our knowledge for treatment and knowing the pathophysiology of a disease. This goal is impossible to achieve without doing a retrospective analysis of biomarkers through clinical trial studies.

Energy restriction

Calorie energy restriction

Continuous energy restriction (CER) has long been studied as an approach for reducing weight and improving metabolic complications (147). However, due to poor compliance and high drop-out rates (148), intermittent fasting (IF) has been conducted as an alternative to CER. For IF intervention studies, participants are asked to restrict their energy intake to only 500-600 kcal per day, for 2 days per week. For the rest of the week, dietary consultation was utilized for healthy eating without eating beyond baseline requirements. It is worth noting that IF intervention studies yield a comparable outcome with CER studies (149, 150).

Caloric restriction is the fundamental basis for weight loss. The early remission of diabetes following bariatric surgery is largely thought to be due to severe caloric restriction (151, 152). However, long term maintenance of restricted caloric intake without bariatric surgery is extremely difficult, resulting in failure of most cost-effective lifestyle programmes (153, 154). Whilst long-term, daily, very low caloric restriction is unsustainable, intermittent reduced intake may be more achievable with similar dramatic benefits in metabolic health as bariatric surgery (155). Intermittent fasting has been practiced by various religious groups for centuries and may be equally accessible across those of different socioeconomic and cultural backgrounds. Freedom to choose the types of food to be consumed has been shown to be important in long term adherence to nutritional advice, hence a self-selected meal plan for intermittent fasting days is encouraged. A meal plan that is adaptable to the current usual dietary pattern and takes into account cultural preference and budget choice of participants, as well as freedom to choose the two fasting days in each week is also advised. Whether such intermittent fasting is an acceptable and effective technique in preventing progression of pre-diabetes is currently unknown.

Alternate day fasting

Alternate day fasting involves alternating fasting days, during which no calories are consumed, and feeding days, during which foods and beverages are consumed ad libitum. In 2007, Varady and Hellerstein (156) reviewed alternate-day fasting studies in rodents and concluded that this fasting regimen was as effective as simple caloric restriction in reducing obesity-associated body weight, fasting insulin and glucose concentrations. Alternate-day fasting in rodent models of obesity has also been shown to reduce total plasma cholesterol and triglyceride (TG) concentrations, reduce liver steatosis and inflammatory gene expression, and have beneficial effects on cancer risk factors, such as cell proliferation (156-159).

Four intervention studies have explored the metabolic effects of alternate day fasting (160-163). Sample sizes were modest and ranged from 8 to 30 normal weight adults and 10 overweight or obese adults (160), but no information was provided about the physical activity levels of these participants. Two of three studies reported significant weight loss, although the clinical relevance of weight loss in a 1-day study is questionable (163). In the 22-day study of alternate-day fasting, participants experienced a mean weight loss of 2.5 % ($p < 0.001$) (162). Three of the studies found a significant decrease in at least one glucoregulatory marker. In contrast, the study that included overweight and obese participants did not, and, in fact, reported a detrimental effect of 1-day total fasting on postprandial glucose and insulin the following day (160). This study by Antoni et al.(3)and Halberg et al.(4) examined lipid levels with mixed results. The 1-day fasting study observed improved postprandial triglycerides (TGs)the following day, whilst the other study observed improvements in high-density lipoprotein (HDL) cholesterol and fasting TGs, but increased low-density lipoprotein (LDL) cholesterol at the study's end point. One of two studies assessing inflammation found significant improvements in inflammatory biomarkers.

Of the four studies, one study had enrolled normal weight adults in which there were no substantial improvements in metabolic risk factors. Although not a focus of this review, hunger, mental status, and post-fast energy intake are important outcomes to consider with extended fasting during

waking hours. Appleton and Baker (164) recently reported that in women (n = 16), a 2-day fast resulted in distraction but not hunger, and was associated with lower mood and perceived work performance compared with 2 days prior to and following the fasting period. Antoni et al. (160) observed that a 1-day fast resulted in a 30 % reduction in energy intake 3 days post-fast. Heilbronn et al. (162) noted that participants reported considerable hunger on fasting days, which did not decrease over time. The sparse data on alternate day fasting suggests that this regimen can result in modest weight loss and lead to improvements in some metabolic parameters. However, reports of extreme hunger while fasting indicate that this may not be a feasible public health intervention.

Probiotics

Probiotics

Gut microbiota (microorganisms colonizing our intestines) have emerged as a novel mediator of obesity and T2D, through modification of dietary energy extraction, hunger stimulation, inflammation and glucose and lipid metabolism (165, 166). The most compelling evidence for a causal role of gut microbiota in obesity and metabolic disease comes from both animal and human experiments in which faecal transplants were able to alter recipient body weight and insulin sensitivity independent of energy intake (167, 168). Probiotics defined as "microorganisms able to confer defined health benefits to the host," work in complex and poorly understood ways to alter macro and micronutrient availability from the gut (169), modify lipid and cholesterol metabolism (170) and exert anti-inflammatory responses (171), often in a microbe strain-specific fashion.

Probiotics Supplementation

The effects of probiotics in diabetes are strongly encouraging. The combination of *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* BB12, (10^{10} colony forming units each daily) was successful in reducing the incidence of gestational diabetes from 36 % among women given placebo to 13 % in those who received the probiotics combined with dietary advice (64 % decrease) in a randomised controlled trial among 256 Finnish pregnant women (172). There was a 144 g reduction in birthweight and also a reduction in maternal adiposity 6 months postpartum with probiotic

treatment (172). Other types of *Lactobacillus* probiotic supplementation have been demonstrated to improve insulin sensitivity (173) and reduce body weight (174) outside of pregnancy and to result in greater early weight loss after GBP surgery (175). *Bifidobacteria* concentrations have been associated with weight reduction (176). *Bifidobacterium* genera are healthy commensal bacteria, which are less common in the gut microbiome of obese mothers and those who gain excessive weight in pregnancy (177). In the double-blind, randomised, placebo-controlled Probiotic in Pregnancy (PIP) study of *Lactobacillus rhamnosus* HN001 (**HRC 11/318),** 423 women with a personal or partner history of atopic disease, who were randomised to receive daily HN001 ($6x10^9$ cfu) or matching placebo, the incidence of GDM was significantly lower in the HN001 group. This was most significant among high-risk women, defined as obese, of older age or with previous GDM.

Links between Probiotics and obesity and diabetes

Probiotics and obesity and diabetes

There is growing evidence linking gut dysbiosis with obesity and diabetes. Animal studies have suggested that probiotics can improve insulin sensitivity and beta-cell dysfunction by regulating the key signaling pathways (178, 179). Specific strains from the Lactobacillus and Bifidobacterium genera have been named the most effective for glucose and weight control (180). Recent evidence from a New Zealand study showed that presence of the probiotic *Lactobacillus rhamnosus* HN001 can result in improved gestational diabetes prevalence despite no changes in body weight (181). Other research has linked probiotics to improvements in inflammation (182) and other biomarkers of oxidative stress (183). Multiple studies have investigated the synergistic effect of a probiotic intervention with a hypocaloric diet, and have reported augmented improvements in health markers (184-186). However, no studies have examined the effect of probiotics in combination with intermittent fasting.

Biomarkers

Haemoglobin A1c (HbA1c) or Glycated hemoglobin

Identification of community populations at increased risk of T2D must be an important part of any disease prevention strategy. Developing simple public health screening methods that effectively identify individuals who are at a greater risk of T2D is extremely important, as it allows timely intervention both to delay or prevent progression. Taking a measurement of glycated haemoglobin (HbA1c) -- a longer term marker of high blood glucose concentration -- is a simple and cheap method that has been adopted by the American Diabetes Association (ADA) within the past 10 years (187) for diagnosis of T2D. Glycation of haemoglobin (Hb) occurs throughout the typically 120 day lifespan of a red blood cell, which means the relative proportion of HbA1c at any one time depends on the mean circulating blood glucose level over that 3 month period. Use of HbA1c as a screening marker has rapidly been adopted in clinical practice by a growing number of countries where it may provide an excellent cost-efficient approach to T2D screening, providing it is shown to have adequate sensitivity and specificity. Comparison of costs from the National Institute for Health and Clinical Excellence (NICE) UK, for example, showed the indicative cost of HbA1c test to be half that of an OGTT (GB£4.04/US$5.05 vs. GB£7.48/US $9.34) (188) when widespread testing was adopted in 2011 (189). Whilst there is evidence that it may be a good marker of T2D, there is considerably more controversy as to whether it may also correctly identify those pre-diabetic individuals with increased future risk of T2D, but yet without the full disease (190).

Body Composition Measurement

The significant impact of body composition is to assess physical utility characteristics such as activity and nutrition. There is no doubt about the effect of excess fat on the onset of chronic diseases (191). Additionally, there are several ranges of methods available for the assessment of body composition, and they are categorised according to the number of compartments that they measure. A two-compartment method quantifies fat, and fat-free mass, while three-compartment methods measure fat mass and two compartments of fat-free mass. Each method needs to be evaluated according to costs, validity or reliability, applications, risks and availability (192). There

are three types of classifications for body fat: fat mass (FM), which includes total extractable fats; fat-free mass (FFM), which includes all chemicals, water (≈73%), bones (≈7%), internal organs and connective tissues (≈20%), and lean body mass (LBM), which comprises of small amounts of essential fats (193). The body composition assessment is an accurate method to standardise classification of body fitness, and therefore, the applications of body composition include: determination of health risks associated with low or high levels of fat; monitoring impacts of a given nutrition; exercise and intervention; and measuring of body weight to assess growth, development, and maturation.

Ordinary Anthropometric Measurement

Obesity is defined as an excessive amount of body fat in association with body weight (194). Obesity can be a result of excessive energy consumption or alterations in body energy expenditure that leads to a positive energy equilibrium (195). The study of evaluating the human body based on the dimension of bones, muscles and fat tissues (adipose tissues) is called anthropometry. Anthropometric, or body composition measurements, are critical in the context od pre-diabetic monitoring due to their potential to predict or proceed with monitoring the onset of metabolic disorders, such as obesity and diabetes. Anthropometry refers to measuring the size of the body based on values and ratios, including, but not limited to, height to weight (BMI), the waist to hip ratio (WHR), as well as neck and femur circumferences. Circumferences, in particular, are vital to record during anthropometry measurement, requiring the use of tape measurement, and a consistent placement on the same position for all subjects to increase validity and reliability of the measures.

While the BMI measurement is universally accepted as an indicator of obesity, it is not the best indicator of obesity, for instance, older people have more body fat at any given BMI compared with younger people. A low BMI does not always mean there is less body fat (196), therefore, it is crucial to measure other indices of body composition. For example, LBM is increased by obesity (197) and neck circumference measurements reflect upper-body fat indirectly (198). High proportions of WHR is a common calculation used to predict the risk of T2D (199), working as a

crude scale of fat distribution as waist circumference has a direct relationship with abdominal content, musculature, subcutaneous adipose tissue (SCAT) and visceral adipose tissue (VAT) (200). However, a recent clinical trial studying obese patients has shown that there is a direct correlation between neck circumference and metabolic diseases, and thereby, that neck circumference information is superior to waist circumference data (201). The waist circumference measurements are also considered to be the surrogate markers used to predict metabolic syndromes (202). Of note, it is accepted that neck circumference cut-off values of 36 and 39 cm or greater in males and females respectively is associated with increased Homeostatic model assessment of insulin resistance (HOMA-IR) and Hemoglobin A1c (HbA1c) (201).

Dual Energy X-ray Absorption

Dual-energy X-ray absorptiometry (DXA, previously DEXA) is a three-compartment method for the evaluation of body composition due to its abilities to measure bone mineral, fat and fat-free mass. DXA is also able to measure total body bone (TBB), total body fat (TBF), bone mineral content (BMC) and bone mineral density (BMD). DXA can also be replaced by dual photon absorptiometry (DPA). Instead of a radioisotope, X-rays are used in this method because X-rays are more accurate than DPA (203). DXA is a straightforward and non-invasive procedure for the assessment of body composition. In DXA only 1 to 3 milliradians of x-rays are used. The principle of DXA procedures is based on the reduction of X-rays with high and low photons. Chemical components and the density and thickness of tissues are measurable from pixels created by the instrument (204). Nowadays, bone densitometry is a favourable method to diagnose osteoporosis. In practice, the best method to evaluate bone density functions is measuring BMD, and is most reliably measured using DXA on spine and femur samples (205). DXA is a very precise method to assess body composition (206) and its use in biomedical research is quite new. Body composition plays a major role in the assessment and management of different diseases (207), and the increased prevalence of obesity and its comorbidities have accelerated curiosity in the utility of straightforward and accurate technologies such as DXA for the body composition evaluation (208).

Bioelectrical Impedance Analysis

Bioelectrical impedance analysis (BIA) is a two-compartment method, non-invasively performed by attaching electrical wires to the body to measure resistance (R) and reactance (Xc). Both R and Xc are used to quantify impedance (Z), phase angle, total body water (TBW), FFM, FM and body cell mass (BCM) values (209). In BIA, resistance to an electrical current is negatively related to the distribution of TBW and electrolytes (192). The principle of BIA is based on the fact that different body components have different resistance to the passage of electrical currents (210). An electrical current is used to measure resistance as opposition to the electrical current through the body, and reactance measures values originating from cell membranes. Resistance and reactance are based on electrical current and are capable of measuring FFM (211). Adipose tissue and bone minerals have greater resistance to current flow than FFM, as FFM includes less water (210). About 73 % of the body's FFM is water, hence why total body water is estimated, and FFM is calculated based on water (212). The higher conductivity, or low resistance, of lean tissues is due to a large amount of water and electrolytes, while bone, skin and fat, have low high resistance (low conductivity) due to their lower amounts of water and electrolytes (211). Normally four electrodes are used for BIA measurements. With the patient in a horizontal position, two distal wires for the wrist and hand, and another two proximal wires for the ankle and foot are used with low electrical current (500 to 800 μA) in BIA (192). Because BIA has been identified as an accurate procedure to measure body fat, it can be used instead of DXA (213).

Energy Expenditure

Energy expenditure (EE) is defined as the number of calories consumed during a distinct time, often 24 hrs. The resting energy expenditure (REE) is a common method to represent EE. The proper range of REE is 1800-2200 kcal/24 hr (214). Daily energy or calorie consumption is quantified by energy expenditure in a resting position. Several factors such as disease states, age, and being obese, influence the calculation of EE (215). Measuring EE during a chronic disease state is important, as it can be helpful to prevent overfeeding in individuals during an intervention

screening. REE directly aims to manage patients' care. It has been shown that obese females have higher EE in comparison with females with normal weight, which may be related to the higher amount of FFM in women (216). Furthermore, there is a significant and positive correlation between EE and body size (197). Indirect calorimetry is used when EE is measured from VO_2 production because heat cannot be measured directly (217). Indirect energy expenditure analyses respired gases, namely oxygen uptake and carbon dioxide expression from the lungs. The respiratory quotient (RQ) in a person is calculated as the ratio of exhaled CO_2 to consumed O_2. RQ is a popular index of EE (218). RQ values indicate consumed fats, carbohydrates and proteins being used for energy. An RQ within the range of 0.65 to 1.25 is defined as a stable-state condition, while values below or greater than this range is considered to be a health issue. The RQ value also represents carbohydrate metabolism (RQ=1.0), lipid metabolism (RQ=0.71) and protein metabolism (0.80) (214). Once the rate of metabolism is slow, a person has difficulty managing weight loss. A significant benefit of REE measurement is that it monitors metabolic rate to avoid further unnecessary analysis. In other words, a given ratio and value from REE aims to evaluate the success of strategies for losing weight (219). Additionally, monitoring REE and RQ is an easy way to check whether or not a person is following a given intervention appropriately (220).

Magnetic resonance imaging

Magnetic resonance imaging (MRI) is well-suited for fat quantification (221-223) because it is non-invasive and provides sensitive mechanisms for differentiating fat from lean tissues based on T1 relaxation and chemical-shift properties. Furthermore, it does not utilize ionizing radiation, has unlimited repeatability, and is safe for use in children. Magnetic resonance spectroscopy (MRS) is generally considered the clinical gold-standard non-invasive technique for *in vivo* fat and metabolite quantification. It is routinely used for measuring liver fat (224-226), myocellular lipids (227), tissue composition (228), and brain metabolites. In 1984, Dixon (229) described a two-dimensional imaging approach that exploited the chemical-shift difference between protons in water and fat to separate the two moieties. Over the past 25 years, Dixon's method has evolved significantly (230-232) and recent advances in reconstruction algorithms have led to the

development of a fat-water three-dimensional (3D) imaging technique called IDEAL (Iterative Decomposition with Echo Asymmetry and Least squares estimation) (233, 234). IDEAL is particularly robust to resonance offsets (off-resonance due to magnetic field nonuniformity, a common system imperfection), and produces separated fat and water images that are optimal in terms of signal-to-noise ratio (235).

ImageJ for fat quantification by MRI

National institute of health (NIH) has released a free-ware medical imaging software program (NIH ImageJ; NIH, Bethesda, MD), an open source Java-written program that is now at version 1.31 and is used for many imaging applications, including those that that span the gamut from skin analysis to neuroscience. ImageJ is in the public domain and runs on any operating system (OS). ImageJ is easy to use and can do many imaging manipulations (236).

Metabolic syndrome and body fat distribution

Definition of Metabolic syndrome

Metabolic syndrome (MS) was defined using the modified definition outlined in the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) guidelines(237) following the waist circumference (WC) criteria proposed by the WHO's Regional Office for the Western Pacific Region (238): (1) WC ≥ 90 cm in men and 80 cm or more in women; (2) fasting TG ≥ 150 mg/dL or drug treatment for elevated TG; (3) HDL cholesterol < 40 mg/dL in men and less than 50 mg/dL in women or drug treatment for low HDL cholesterol; (4) blood pressure(BP) ≥ 130/85 mmHg or taking antihypertensive medication; and (5) fasting glucose ≥ 100 mg/dL or taking antidiabetic medication.

MS is characterized by clusters of metabolic abnormalities and has been associated with the incidence of T2D as well as cardiovascular morbidity and mortality (239, 240). The increasing prevalence of obesity and T2D has led to an increased prevalence of MS, which has a substantial

impact on public health (241, 242). Therefore, identifying subjects who have a high risk of MS is important in clinical practice as early intervention via lifestyle modification may prevent the incidence of MS and thereby reduce clinical burden.

Based on previous studies, regional body fat distribution, regardless of general obesity, may play a critical role in MS (243). WC, as a component of MS, does not sufficiently discriminate between visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT), and has shown stronger associations with SAT than with VAT (244). Both VAT and SAT contribute to abdominal obesity, however, there has been debate regarding their effect on MS. Several cross-sectional studies have reported a relatively consistent relationship between VAT, MS, and insulin resistance (245-248), but the association between SAT and MS remains controversial (245-247, 249-252). While VAT involves an active endocrine organ that releases numerous adipokines and hormones that regulate metabolism and inflammation, SAT may be protective against the development of metabolic abnormalities as a 'metabolic sink' (253).

Abdominal fat quantification by MRI

The visceral adipose tissue (VAT) surrounding the abdominal organs contributes to central obesity and has been described as an inflammatory endocrine organ associated with negative health consequences (254, 255), including elevated risk for cardiovascular disease and diabetes (256). Subcutaneous adipose tissue (SAT) is not as strongly linked with metabolic disease (257); however, the VAT/SAT ratio has been cited as a predictor of mortality and cardiac events independent of total VAT volume (258). The distribution of fat stored in the VAT, SAT, and specific organ depots such as the liver, has a prognostic value superior to surrogate measures such as body mass index (BMI) or waist circumference (255). It is therefore important to carefully consider the limitations and differences between current methods used to measure VAT and SAT storage depots.

CHAPTER THREE

MATERIALS AND METHOD

Study Design

The current study was a single-center, prospective, randomized, double-blind study.

Ethical Approval and Ethical Consideration

Ethical Approval was received from New Zealand regional ethics committee (16/STH/107). This study was prospectively registered at ANZCTR (ACTRN12611000751976) The information sheet of this study was provided to the subject to decide to join in this study (Appendix A). For all the confirmed subjects for the bariatric surgery, a written informed consent was obtained (Appendix B).

Study Samples

Obese, prediabetes patients of Maori, Pacific, NZE or Indian ethnicity. Recruitment was commenced in July 2016 with the goal of achieving complete recruitment of the first tranche of 44 participants in December 2016 randomised to groups 1 and 2 (HRC feasibility Grant awarded for this). The remaining participants was recruited subject to further grant funding.

Location of Sampling and Measuring

All samples were collected at Body Composition Unit, Auckland City Hospital. Although the anthropometric measurements were recorded and kept in the Body Composition Unit for further analysis, human plasma samples were aliquoted and transferred to the School of Science lab, AUT University for analysis.

Sample size calculation and power

Auckland has at least 25% prevalence of prediabetes in the adult population. To allow for 10% loss to follow up, we recruited 560 participants with prediabetes. A total sample size of 504 people with prediabetes (126 per arm), provides 90% power to detect a minimum effect size of 2.5mmol/mol difference in HbA1c in one of the 3 intervention strategies: arms (1), (2) or (3), relative to control

arm (4), using the Dunnett (with control) multiple comparison test at a 5% significance level (PASS 13, version 13.0.4). The standard deviation for change in HbA1c is assumed to be 4 mmol/mol as reported in another prediabetes nutritional intervention study(281). The effect size of 2.5mmol/mol has been chosen to be the minimum clinically significant reduction in HbA1c among patients selected to have prediabetes. This sample size was also yield 90% power to detect an interaction effect of 2.3mmol/mol between intermittent fasting and probiotic.

Statistical Analyses and Data Management

Statistical advice has been provided on the study design, sample size and planned data analysis from Associate Prof Lindsay Plank, who is a member of the research team.

Binary endpoints were analyzed using logistic regression to estimate odds ratios of treatment groups compared to the control group. Continuous outcomes were modelled using generalized linear models to estimate any changes in outcomes with the treatment group compared to the control group. Multivariable analyses were control for potential confounders including BMI and ethnicity. Outcomes compared across all groups was adjusted for multiple comparisons.

Randomization

Participants were allocated to treatment groups by randomization with minimization based on age (grouped as 20-30, 30-40, 40-50); time since diagnosis of T2D (grouped as <5yrs, 5-10yrs, >10yrs) and ethnicity (grouped as Māori, Pacific, European, or other). Computer with allocation concealment performed randomization.

Inclusion and Exclusion Criteria

Inclusion Criteria

Adults with prediabetes based on HbA1c 40-49mmol/mol, and BMI >30 kg/m^2 (>27 kg/m^2 in those of Indian ethnicity) and able to provide informed written consent

Exclusion Criteria

Participants who have the aged lower than 18 or greater than 65 years-old were excluded. Pre-existing diabetes, previous bariatric surgery were also considered as limitations for the study. Subjects with a BMI of greater than 40kg/m^2, in which the fitting on MRI scanning would be difficult, people who is taking on glucose lowering medications, any conditions that might influence body weight regulation (eg: malabsorption, thyroid disorders, eating disorders were excluded from study participation,

Being used of systemic steroids, excess alcohol intake (>21 units per week in men, >14 units per week in women) were also asked to refrain from this study. All subjects had been asked to check whether that have had stable body weight in previous 6 months, no any planned for any major changes in physical activity during the study to an extent that might interfere with the study outcome. Any participants who had blood donation within past 2 months prior to the study (and at the endpoints) or adults with a weight change of greater than 3kg within 3 months prior to first baseline visit had been removed. Other indications for excluding participants from the study were psychiatric disease, pregnant women, or lactating, or intending to become pregnant within the study duration, significant renal disease (GFR<30), congestive heart failure, unexplained syncope, recent myocardial infarction or stroke within 6 months, porphyria, thalassemia (or other blood disorders in which HbA1c is inaccurate for glycemia) and splenectomy. The final criteria were any participation in other clinical studies within the previous 6 months or not accepting of 5:2 intermittent fasting or probiotic supplementation.

Study Design and Procedures

This was a randomized placebo controlled parallel 4 arm trial designed according to Consolidated Standards of Reporting Trials (CONSORT) guidelines (282). We investigated the role of an oral probiotic capsule consisting of *Lactobacillus rhamnosus HN001* at a dose of 6x10^9 colony-forming units per day each, or placebo along with either 5:2 intermittent fasting or standard dietary advice. Eligible participants were randomised to 1 of 4 arms: (1) intermittent fasting + probiotic (2) intermittent fasting + placebo (3) healthy eating + probiotic (4) healthy eating + placebo (control).

Randomisation will be stratified by ethnicity (Māori, Pacific, Indian, NZ European) and BMI category (<35 or ≥35 kg/m^2). 560 patients with prediabetes were randomized into one of these 4 groups (**Figure 3.1**).

Engagement of Patients with Prediabetes

We had a multi-pronged approach to optimize recruitment including through primary healthcare organizations, general practitioners, practice nurses, community contacts, advertising in print media and on community radio. Analysis of the Diabetes Projects Trust (Auckland) database suggests that there were over 18,000 patients recorded as having prediabetes. A greater number of patients with prediabetes were selected from MedTech queries run through ProCare and East Tamaki Health Care practices, who was invited to take part in this study. The research assistant arranged a screening visit to explain the study, confirm eligibility and obtain informed consent.

Baseline assessments

After screening, confirming eligibility, and obtaining consent, comprehensive information were obtained from patients and their clinical records, including:

(1) Demographic, socioeconomic, educational and occupational data
(2) Medical history
(3) Family history of diabetes, hypertension and cardiovascular disease
(4) History of smoking, alcohol and other drug use
(5) Medications and nutritional supplements
(6) Anthropometrics: weight, height, skin folds, waist circumference
(7) Blood pressure
(8) Collection of fasting blood, fecal specimen for biobank (analysis subject to additional funding), 5 time point OGTT, DEXA
(9) MRI/MRS (subset of patients only)
(10) Questionnaires include the 12-Item Short-Form Health Survey (SF-12) (283) and the 7-day food diary either on paper or electronically using the Easy Diet Diary app: http://easydietdiary.com/

Biological samples (blood, and fecal specimen), body composition assessment was obtained at baseline and at 3 months and 12 months. A subset was invited to have a MRI/MRS scan using 3 Tesla Siemens machine at baseline and 3 months, directly before their 2 hour OGTT assessment. An indwelling intravenous catheter was inserted into one antecubital vein for 5 time point sampling during the 75 g oral glucose tolerance testing. After the fasting samples was collected into P800 tubes (for stability of gut hormone analysis), participants were moved to the indirect calorimetry area where they were asked to lie down in the supine position for basal metabolic rate measurements. After completion of indirect calorimetry to measure RMR, they were asked to consume 75g oral glucose drink (Carbotest, Thermo Fisher Scientific). DEXA will be performed using iDXA (GE-Lunar, Madison, WI). Following this scan, blood draws at 15min, 30min, 1 hour and 2 hours were made. All samples were centrifuged and the supernatant plasma were stored in the -80 freezer until required for analysis. Participants was asked to collect a fecal sample either during their body composition assessment visit or in advance and kept frozen until delivery to the unit, after which they were stored in the -80 freezer until required for gut microbiota analysis.

Measurement of liver and pancreatic fat:

The 3 Tesla Siemens MRI system was used with a sagittal localizing image acquired. A 3D dual gradient-echo sequence was acquired water/fat images in one acquisition using a 2-point Dixon technique with a T1-weighted spin echo pulse sequence (TR of 6.5 msec and a TE out of phase/in phase, 2.4/4.8 msec, flip angle 12 degrees, matrix 256 x 128, and 0.7 number of excitations). After the 60 second, fast abdomen scan, the pancreas is located and a number of 3.0mm slices were planned to cover the entire pancreas. Similar 2-point Dixon protocol was used. Visceral fat and subcutaneous abdominal fat as well as fat depot in pancreas were distinguished through image processing calculations. After the abdominal scan, the patient was asked to stay in the scanner for a further 12 minutes and MR proton spectroscopy was performed to determine the liver fat content. MRS of the selected voxel was performed and the spectrum recorded using the stimulated-echo acquisition mode sequence; echo time of 20 msec, a TR of 3000 msec, a mixing time of 30 msec,

1024 data points over 1000 kHz spectral width with 32 averages. Water-suppressed spectrum with 128 averages was also recorded to detect weak lipid signals. The total time for the participant in the MR examination room was estimated at 40 minutes.

Randomization

Randomization was managed using a web-based protocol, with random number codes. Participants were stratified by ethnicity and BMI category (BMI of 27-35 or BMI >35 kg/m^2) and randomly allocated to one of the four study groups (**Figure 3.1**).

Overview of recruitment and randomisation

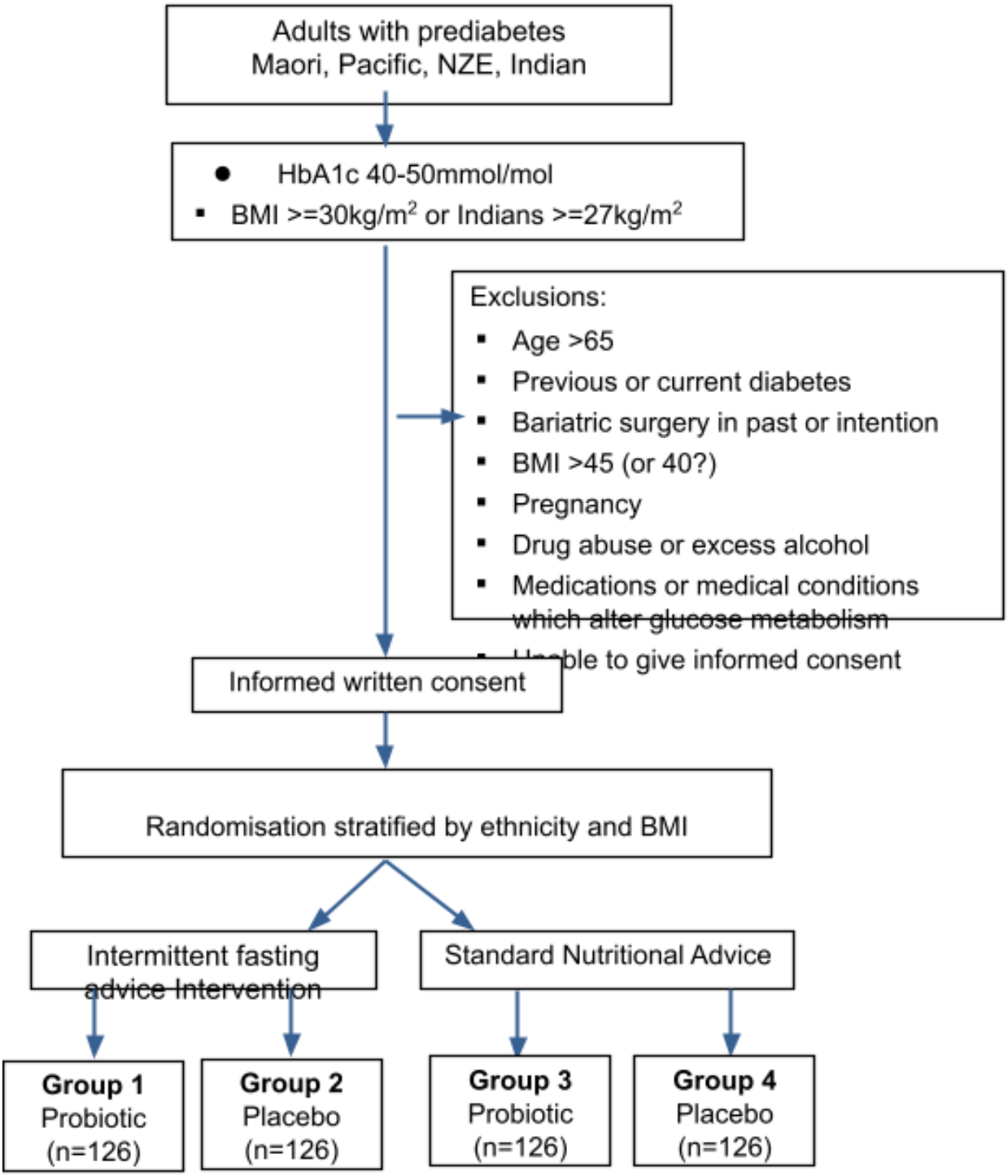

Figure 3.1 Overview of recruitment and randomization

Probiotics and Placebo

The oral probiotic capsules contained *Lactobacillus rhamnosus HN001 (Fonterra)* at a dose of $6x10^9$ colony-forming units per day each. This was the same dose that was used in the probiotic in pregnancy study which reduced the outcome of gestational diabetes. The identical placebo capsules comprise microcrystalline cellulose and dextrose anhydrate and were also supplied by Fonterra.

Allocation Concealment, Blinding and Compliance

Placebo and probiotic were identically packaged. All clinical and research staff and participants were masked to the randomised probiotic/placebo allocation. Although it was not be possible for clinical and research staff to be blinded to the nutrition group allocation, the key health outcomes including results from the HbA1c, OGTT, and DEXA and MRI/MRS were ascertained by those who were not aware of the allocation. Compliance with probiotics/placebo was assessed by patient self-report and checking blister packs by the research team.

Proposed of the study

We proposed a single-centre, randomized, placebo controlled dietary intervention study examining the relative efficacy of an intermittent fasting diet, with or without a probiotic supplement, to prevent progression of prediabetes. We have chosen two potentially cost-effective interventions targeting the gut microbiota which may be easily translated into primary care management pathways for prediabetes.

Significance of study

Intermittent fasting and/or probiotic supplementation interventions will: 1) improve glucose metabolism (reduced HbA1c at 1 year); 2) reduce body weight; and 3) reduce other obesity related complications

Study hypothesis

We hypothesized that in overweight and obese adults with prediabetes, intermittent fasting and/or probiotic supplementation interventions will: 1) improve glucose metabolism (reduced HbA1c at 1 year); 2) reduce body weight; and 3) reduce other obesity related complications

Study Groups

Intermittent fasting interventions (groups 1 and 2)

These two groups received advice on calorie counting in order to achieve significant calorie restriction on two out of every 7 days. On the remaining non-fasting days, no specific dietary instruction had been provided other than the standard health dietary pamphlet as below.

Standard Healthy diet intervention (groups 3 and 4)

These two groups received general healthy nutritional guidelines as per the pamphlet produced by the NZ Ministry of Health which contains dietary advice for prediabetes that follows current New Zealand nutrition guidelines.

Encounters with nutrition advisor:

A dietitian or trained facilitator overseen by a registered dietitian provided 4 group sessions of fortnightly support around self-selected meal plans for intermittent fasting days that were adapted to the participants current usual dietary pattern and takes into account cultural preferences and budget choice of participants.

Behaviour Change Techniques:

Behaviour change techniques were incorporated in the nutrition education support including identifying barriers, self-monitoring, goal setting and providing regular feedback.

Physical Activity

Recruited participants were asked to follow a standard physical activity advice. The information for the activities were originated from the Te Wai o Rona Program, accessed though the link;

http://www.sportwaikato.org.nz/te_wai_o_rona_resources.cfm. All participants were asked to follow all key messages of the program

(11) Look for ways to be active everyday
(12) Increase daily exercise
(13) Move more, add more steps
(14) Reduce sedentary leisure time

Optimizing Engagement and Retention

Members of the research team met participants at 3 monthly intervals during the study (**Figure 3.2**) in order to monitor weight, blood pressure, HbA1c, to provide further supplies of probiotic/placebo and check for compliance.

Three month and 1 year research assessments

These appointments were scheduled at the research center. The food frequency and physical activity, questionnaires, and an updated medical history were obtained by the research assistant (**Figure 3.2**). Body anthropometry measurements were recorded, DEXA, RMR, OGTT, and fecal sample was stored.

Research plan - Flow chart for all PROFAST participants

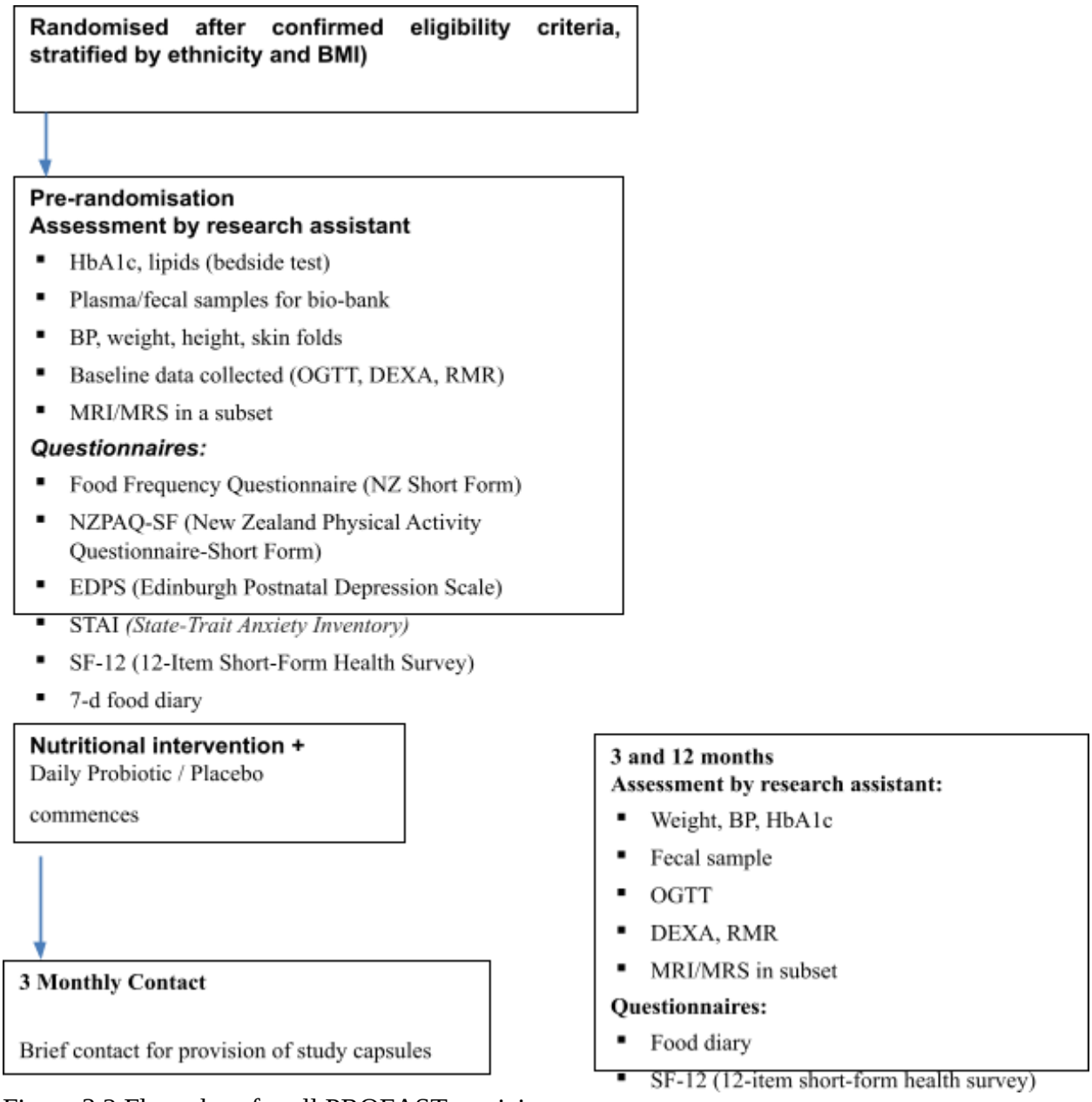

Figure 3.2 Flow chart for all PROFAST participants

Compliance Statements

The study was conducted in line with the Principles of the Declaration of Helsinki (1996) and according to best practice as detailed in the PROFAST trial standard operating procedure manuals.

Before the trial commences, ethics approval was obtained from the Health and Disability Ethics Committees (HDEC), Ministry of Health, New Zealand (http://ethics.health.govt.nz/). The researchers did respect the principles of partnership, participation and protection in the Treaty of Waitangi (the founding document of New Zealand). In addition, the PROFAST study was follow the 2012 *Ethical Guidelines for Intervention Studies* published by the National Ethics Advisory Committee, Ministry of Health (http://neac.health.govt.nz/streamlined-ethical-guidelines-health-and-disability-research).

The PROFAST trial was registered with the Australian New Zealand Clinical Trials Registry. A Universal Trial Number (UTN) will be obtained from the World Health Organization (http://www.who.int/ictrp/unambiguous_identification/utn/en/).

Quality controls and quality assurance

Confirmation of the quality and safety of the probiotic

Fonterra makes the *L rhamnosus* capsules from library colonies which are meticulously DNA fingerprinted to ascertain the presence of *Lactobacillus rhamnosus HN001* only. The packaging and storage of the probiotics were complied with company specifications ensuring the quality of the product.

Data handling

Consent forms and participant data that contain identifying information were stored separately. Most study data were collected electronically on a tablet but when paper forms or questionnaires are completed these were coded using study ID numbers; to ensure confidentiality for each participant. All data forms were stored securely in locked cabinets for 10 years and all identifiable computer data would be accessible only using a password. Access to the data forms and computer data is restricted to researchers directly involved with the study.

Record of Demographic Information

After receiving verbal consent, all the individuals were asked to fill in a survey form which includes the following items: age, height, weight, race, smoking, and alcohol drinking habits, bowel motion, the frequency of defecation and medical history. For the follow-up study, all subjects were asked to express their feeling after drinking the glucose syrup. An example of a demographic questionnaire is attached (Appendix C).

Anthropometric and Body Composition Measurements

Body composition obtained in this study include weight, height, circumference of waist, hip and neck. Weight was measured using an electronic measuring machine. To measure the height, the individuals were asked to take off their shoes, stand with their back against the wall with their feet together. The back on their feet, buttocks, shoulders, and the back of their head is adjusted by the researcher. They were asked to look straight ahead to ensure that their bodies were as straight as possible. Subsequently, their head level positions were marked with a pencil. A measuring tape was then used from the ground up to determine their height; BMI then was calculated using the formula: BMI = weight (kg) / Height^2 (m^2). Circumference measurements were taken at least twice; the measurement accepted if the difference between two measurements were not more than 2 cm. However, the third measurement was done if the difference was more than 2 cm. In this case, the two closest measurements were averaged and recorded for analysis. In this study, neck, hip, and waist circumference were measured as previously mentioned (5). To measure the waist circumference, the subject was asked to lift his/her clothes to put the tape in the appropriate position. The subject was asked to stand erect with the belly relaxed. The tape was positioned in a horizontal position at the nearest part of the torso. For those subjects who were more obese, the area between the ribs and iliac crest was considered to measure the waist circumference. To measure the hip (buttocks) circumference, the subject was asked to stand erect with arms at the sides and feet closed together. The tape was positioned around the hip in a horizontal position without pushing the skin. Following waist and hip measurements, the WHR was calculated according to a guideline from WHO(6).To measure neck circumference, the subject was asked to

put off any clothes from his/her neck. The subject sat erect. The tape was positioned around the neck just inferior to Adam's apple with the least amount of pressure on the neck. The measurement was taken promptly to avoid any discomfort for the subject (284).

Bioelectrical Impedance Analysis

BIA, which is a rapid, non-invasive and inexpensive method, was used to measure body composition. Different procedures employed in BIA are dependent on various devices available. Fat is a poor conductor of electricity while a tissue which is free from fat (or FFM) is a good conductor of electricity. Therefore, the resistance to the electric flow is used to determine the proportion of body fat as a percentage. Measurement by BIA is a quick procedure, normally taking less than a minute. However, care should be taken during BIA. In lean people, the percentage of fat is overestimated, while in obese people it is underestimated. Since the result of BIA is directly related to the amount of water in the body, the hydration state of an individual can significantly affect the outcomes. In order to address hydration as a factor, before doing BIA, all subjects were asked to abstain from exercise and drinking alcohol for at least 8-12 hours. BFM and FFM were measured by BIA. The Body Stat Quadscan 4000, UK instrument was utilised in this study. The impedance (Z) is measured through low-level electrical current pass through the patient's body using a BIA machine. Four electrodes were attached to the hand, wrist, foot, and ankle. An excitation current 200-800 uA at 50Hz was applied at the distal electrode on the hand and foot to detect impedance on the wrist and ankle. The patient was asked to lie supine on a non-conductive bed. Attachment of the electrodes began by first cleaning the attachment sites with alcohol. Proximal electrodes were placed on dorsal surfaces of the wrist and ankle. The distal electrode was placed close to the second or third metacarpal-phalangeal at hand and foot. The distance between proximal and distal electrode was at least 5 cm. Finally, lead wires were attached to the electrodes. Red leads were connected to the wrist and ankle and black leads attached to hand and foot. Once leads were connected to the body, the device showed the amount of BFM and FFM.

Dual-Energy X-ray Absorptiometry

TBF, BMC, and BMD were measured by DXA (model iDXA, software V.15, GE-Lunar, Madison, Wisconsin, USA) according to the previous method {Peng, 2007 #1428}. The DXA machine was calibrated according to the manufacturer's calibrator protocol. After measuring the patient's height and weight, patients were asked to lie in a supine position on the scanner bed for head-to-toe measurement. The scanning took approximately 20 to 40 minutes.

Resting Energy Expenditure

Resting energy expenditure (REE) was determined through an open circuit indirect calorimetry device (Deltatrac Metabolic Monitor MBM-100, Datex Instruments, Helsinki, Finland) as previously described {Plank, 2001 #1429}. Each time 30 min prior to the start of measuring, the device was turned on to warm-up. According to the manufacturer's manual, a calibration test was performed to check the function of the instrument. To do REE, all subjects either before or after bariatric surgery were asked to lie in a supine position on the bed, and an oxygen mask was put on the subject to measure REE. This method took about 30 min to perform. REE is a straightforward and safe method to measure energy consumption. To calculate REE, the following formula was utilised as previously described {Plank, 2001 #1429}.

$$REE_{(kcal/d)} = 16.85 * FFM_{corr} + 725$$

where FFM_{corr} is FFM in kg of the subject corrected for abnormal hydration.

Research Method

Blood Samples

All subjects were asked to fast overnight (at least 12 hours). Blood was collected between 8 and 10 a.m. The blood sample was collected after the study subject had rested for 10 minutes. An

intravenous cannula was inserted into the targeted vein to minimize the discomfort of repeated blood sampling, and 20 ml blood samples were drawn every 30 min up to the final sample at 120 minutes. To perform the oral glucose tolerance test (OGTT), each patient was asked to drink a bottle of glucose drink (75g glucose in 300ml water). After 15 min of drinking the solution, the first sample was collected. Plasma separation would be carried out at 4500xg for 10 min at -4°C centrifuge and plasma was aliquoted into a clean tube for further analysis, before being put in -80°C for storage.

MR imaging protocol

Participants were positioned on the table in a supine position then moved into a 3 Tesla Siemens MRI system (Siemens Magnetom Vision, Erlangen, Germany). Sagittal localizing images were acquired from diaphragm to pelvis. The same protocol was used for both visit to ensure the position-matched between visits. Phase encoding was in the anteroposterior direction to minimize the effects of motion-induced phase artefacts and signal averaging (four) was applied to reduce the effect of motion-related artefacts. In addition, respiratory gating was used to combat motion induced artefacts and to reduce the blurring of fat boundaries in the anterior region of the abdomen. A 3D dual gradient-echo sequence acquires water/fat images in one acquisition using a 2-point Dixon technique. The actual scan time for abdominal fat assessment in each patient was less than 40 minutes.

For pancreatic fat, the scan parameters were 3D matrix size 512×512×168 pixels, FOV 357×440, flip angle 9 degrees, patient position; HFS, phase encoding position; COL, TR 5.82, TE 2.46. Slice thickness of 5 mm was applied for covering the entire organ. The slices were acquired during a breath-hold with the same number of slices for both baseline and after intervention scans. Fat and water images have acquired separately.

Magnetic resonance for intra-organ fat content

To achieve the MRI data, a separate MRI axial scan of the abdomen was performed. Liver fat and pancreatic fat were scanned using 3-point Dixon method in which three gradient-echo scans with

adjacent out-of-phase and in-phase echoes were set. The other parameters were TR=50 ms, TE=3.45 ms, averages for 4.60 ms, and flip angle = 5°, For the bandwidth of the scan, 435 Hz per pixel was utilized.

Field of view was set according to patient size (400–480 × 300 mm), and zero filled to give a resolution of 1.39 × 1.40 mm. 5 mm thick sections were used to cover the pancreas during breath holding. Liver scans were acquired during breath holding to cover the liver with slice thickness 10 mm. A balanced turbo field echo (BTFE) image with 5 mm sections matched to the Dixon scan was acquired during breath holding, to distinguish high signal intensity from vessels with visceral fat with lower intensity signals from the pancreas. BTFE images contain a mixed T1/T2 contrast. This is used to delineate the boundaries of the pancreas from adjacent structures, including the surrounding visceral fat, the splenic vein, the superior mesenteric vessels the inferior vena cava and duodenum. Repetition time/echo time/flip angle = 3.1 ms/1.6 ms/40°, turbo factor 95, parallel imaging factor 2, bandwidth 1,156 Hz per pixel). The field of view and zero filling were matched to the Dixon imaging.

Body fat quantification by ImageJ

Fat and water images were calculated from three-point Dixon data using the built-in reconstruction algorithm of the Philips Achieva software, which assumes a complex signal model with a single fat peak and incorporates T_{2}^{*} relaxation at equal rates for fat and water. These images were saved as DICOM files and exported to a workstation for further processing using MIPAV 7.01 and ImageJ 1.48f (NIH, Bethesda, MD). Fat and water images were split, converted to floating point format, and divided by their respective DICOM rescale slope values so that they could be compared on a consistent intensity scale. Fat and water images from successive scans were concatenated to generate 3D fat and water data sets for the entire thymus. From these, a 3D fat fraction map was calculated voxel-by-voxel. In each axial slice, the margins of the thymus were manually traced on the fat and water images, resulting in a 3D mask.

SCAT and VAT

Visceral and subcutaneous fat were distinguished by using ImageJ software (National Institutes of Health, Bethesda, MD) (http://rsbweb.nih.gov/ij/index.html). The number of slices for each scan varied depending on participant height and pancreas position. Region of Interests (ROIs) within abdominal cavity were defined manually by an operator using ImageJ region-growing tools. Segmentation by using the region-growing tool was used to distinguish SAT and VAT portions. Anatomical landmarks were identified, and adipose tissue beds were labelled and segmented into either SAT or VAT area. Intermuscular adipose tissue was excluded in VAT analysis.

To calculate the organ volume, the total area of each slice was calculated for each value and then summarized to be the whole organ volume by multiplying with numbers of slices of each compartment. The 3D slicer software, accessed through; http://www.slicer.org, was utilized in order to identify the landmark of the abdominal portion in each subject (285). Total SAT and VAT volumes were then calculated. Identical sections from both fat and water images were matched. The area of entire abdomen was measured from water images. while the fat portion was processed for the fat area. Corrected threshold was applied for fat images and then only fat area was measured. After getting fat area, visceral and subcutaneous fat volumes were calculated using the formula: percentage of fat = [(fat volume/total volume) × 100]. The volume of subcutaneous and visceral fat in each slice was calculated using the formula: fat area × slice thickness × number of total slices. Visceral and subcutaneous fat areas at L4-L5 were calculated from the L4-L5 proton density fat fraction map by thresholding and watershed analysis (286).

Pancreatic Fat quantification

For pancreatic fat measurement, 5 mm thick slices from both water and fat images were selected to ensure the coverage of entire pancreas. Identical sections from both fat and water images were matched by an observer (DDP). The area of entire organ was measured from water images while the fat slices was processed for the fat area. Corrected threshold was applied for fat images and then fat area was measured.

To calculate pancreatic fat volume, summation of pancreatic fat area from all slices were performed. For each slice of pancreas, the fat volume was quantified from fat area multiplied with slice thickness. The calculation formula was as following.

Pancreas volume = [(fat volume/total volume) × 100].

In addition, the volume of pancreatic fat volume was obtained by using the following formula.

Pancreas fat volume = fat area × slice thickness × no. of total slices.

The same procedure was repeated for second visit after 12-week intervention study. Two results from both visits then were compared following to statistical testing.

For the conventional method, the ImageJ Polygon tool was used to select a region of interest in the parenchymal tissue of the pancreas head, body, and tail. The region was selected to be as large as possible whilst being clear of the pancreas borders to avoid any possible contamination of surrounding visceral fat.

For MR-OPSY procedure, water and fat scans were used. By applying three Region of Interest (ROIs) at head, body and tail of pancreas, the histogram data was obtained following to the previous study (49). The Oval tool of ImageJ was used to select three regions of interest (~100 mm^2 each) to represent equally the pancreas head, body and tail, the size of selection was chosen after pilot studies to permit easy placement entirely within the pancreas considering the irregularity in pancreas morphology (287). The excel spread sheet was used to exclude pixels outside the range of 1-20 %. The same procedure was repeated for second visit after 12-week intervention study. Two results from both visits then were compared following statistical testing. In view of potential uneven distribution of parenchymal fat between different regions of the pancreas observed in some (288-292) but not all studies(293-297), sampling regions were placed equally throughout the pancreas to avoid possible bias.

Pancreatic fat thresholding

Each image slice through the pancreas is 5 mm thick to permit an adequate signal to noise ratio in the fat fraction images. In order to eliminate potential contribution of non- parenchymal tissue (visceral fat, pancreatic duct or blood vessel) within the selected region, a threshold was applied to both methods by collecting the histogram data within the area of selection and computing the resulted data to exclude pixels values outside the threshold limits which would otherwise contribute to the mean value. Anonymized histological sections of pancreatic parenchymal tissues from people undergoing pancreatic surgery taken from various locations in the pancreas showed adipocyte distribution similar to the upper limit of 20% reported by Pinnick et al (298). Hence, the maximum number of adipocytes clustered within a single voxel of pancreatic parenchymal tissues is estimated to be approximately 4000, and any MRI fat signal above 20% is likely to be due to contamination by visceral fat tissue.

Similarly, pixels almost devoid of fat (<1%) are likely to represent major pancreatic ducts or blood vessels, and these cannot be discriminated on the BTFE image. The main pancreatic duct network is suggested though not segmentable on T2-weighted images. A thresholding range of 1–20% was therefore applied to the original data, and the performance of both methods was compared before and after thresholding.

liver fat quantification

To achieve the liver MRS data, a separate MRI axial scan of the abdomen was performed. Liver fat was scanned using 3-point Dixon method in which three gradient-echo scans with adjacent out-of-phase and in-phase echoes were set. The other parameters were TR=50 ms, TE=3.45 ms, averages for 4.60 ms, and flip angle = 5°, For the bandwidth of the scan, 435 Hz per pixel was utilized.

The fields-of-view and reconstructed resolutions were prescribed as per the BTFE sequence. 12 sections of 10mm thickness were used to image the liver, and 12 sections of 5mm thickness to image the pancreas, during four 17-second breath-holds. Custom MATLAB software was used to

separate the fat and water contributions of the MRI and construct fat fraction maps. The polygon tool in the imaging software Image J(299)was used to define regions of interest within the homogenous pancreas parenchyma on both water images on three central sections of pancreatic tissue. For the liver, regions of interest on five homogeneous sections within the right lobe of the liver were evaluated, avoiding the gallbladder, large vessels and surrounding visceral tissue. The triglyceride content in the images was expressed as a percentage of the total

signal from fat and water. The measurements from the defined sections were averaged. For LF contents, a SIVIC (Spectroscopic Imaging, Visualisation and Computing) open-source software (https://launchpad.net/sivic) was utilized. After the spectral transformation, the spectrum graphs were obtained. Then spectral maps were created. Water and fat peaks were integrated to find the area under the curve (AUC). The fat percentage was calculated as:

$$\text{Percentage of liver fat} = [AUC_{lipid} / (AUC_{lipid} + AUC_{water})] \times 100.$$

Liver fat was expressed as a percentage of liver volume corrected for proton density of water and lipid (300).

Statistical Analysis

Statistical Testing

Data were presented as mean ± standard error of mean (SEM). Differences between visits were shown as difference in fat percentage between the visits. Following tests for normal distribution, differences between visits were evaluated using paired t-test. Correlations between changes in body fat depots and changes in BMI or HbA1c using Pearson correlation. Statistical significance was set with p-value of less than 0.05 using SPSS version 24.0 for Windows (SPSS, Chicago, IL, USA). Graphs were generated by GraphPad Prism version 7.0 for Windows (Graphpad Software, San Diego, CA, USA).

CHAPTER FOUR

RESULTS

Recruitment

This study was conducted between December 2016 and December 2017, as determined by the feasibility grant. 33 participants were randomized into this trial (17 in the probiotic group and 16 in the placebo group), and seven participants were lost to follow-up (two from the probiotic group and five from the placebo group). Therefore, the final analysis included 26 participants, with 15 in the probiotic group and 11 in the placebo group.

Table 4.1 baseline characteristics for participant after 12 weeks IF with and without probiotics

Variable	IF (Placebo)	IF (Probiotic)	All Completers	Non-Completers
No. of participants	11	15	26	7
Age (years)	54.1 ± 6.4	52.9 ± 8.7	53.4 ± 7.6	48 ± 8.8
Gender				
Male *n* (%)	2 (18)	6 (40)	8 (31)	1 (14)
Female *n* (%)	9 (82)	9 (60)	18 (69)	6 (86)
Ethnicity				
NZ European *n* (%)	6 (55)	6 (40)	12 (46)	2 (29)
Māori *n* (%)	1 (9)	3 (20)	4 (15)	3 (43)
Pacific *n* (%)	1 (9)	0 (0)	1 (4)	1 (14)
Indian *n* (%)	3 (27)	6 (40)	9 (35)	1 (14)
HbA1c (mmol/mol)	42.9 ± 2.6	43.1 ± 2.9	43.0 ± 2.7	45.4 ± 3.3
Anthropometry				
Weight (kg)	91.3 ± 13.9	98.6 ± 18.4	95.5 ± 16.8	100.5 ± 15.3
Height (cm)	164.7 ± 7.4	168.4 ± 11.7	166.8 ± 10.1	165.1 ± 11.1
BMI (kg/m^2)	33.6 ± 3.7	34.7 ± 4.9	34.2 ± 4.4	36.7 ± 2.4
Waist (cm)	111.1 ± 11.0	112.7 ± 16.3	112.0 ± 14.1	112.5 ± 10.0
Hip (cm)	116.3 ± 9.8	122 ± 13.1	119.6 ± 12.0	122.5 ± 7.5
WHR	0.96 ± 0.1	0.92 ± 0.1	0.94 ± 0.1	0.90 ± 0.10
Neck (cm)	39.1 ± 4.2	40.9 ± 3.8	40.1 ± 4.0	41.0 ± 4.0

Data are presented as mean ± standard deviation or number of participants (percentage). Abbreviations: BMI, body mass index; IF, intermittent fasting; NZ, New Zealand; HbA1c, glycated hemoglobin; WHR, waist-to-hip ratio.

Demographic data and participant characteristics

All participants had pre-diabetes, with a mean HbA1c level of 43 mmol/mol (range: 40–48 mmol/mol) and a mean BMI of 35 kg/m^2 (range: 29–42 mmol/mol). The baseline characteristics of all participants randomized into this trial are summarized in **Table 4.1**. There were no significant differences between the two groups for baseline data.

Clinical characteristics between visit after 12 weeks of IF intervention

Table 4.2 summarized changes of anthropometric and biomarkers after 12 weeks of IF in both allocations, namely with probiotic supplementation and with the placebo.

Table 4.2 Anthropometric characteristics between two visits after 12-week visit

variable	Baseline visit	12-week visit	p value	n
Mean age(years)	52.3	53.4		
Gender				
Male n (%)	9 (27.3)	8 (30.8)		
Female n (%)	24 (72.7)	18 (69.2)		
HbA1c (mmol/L)	43.4 ± 0.5	41.3 ± 0.4	<0.001	24
Total body fat (kg) (DXA)	42.5 ± 2.3	38.8 ± 2.4	<0.001	24
BMD (G/CM2) (DXA)	1.18 ± 0.02	1.18 ± 0.02	0.174	24
Android fat (kg)	4.3 ± 0.3	3.8 ± 0.3	<0.001	24
Visceral fat (kg) (DXA)	2.1 ± 0.2	1.8 ± 0.2	<0.001	24
Weight (kg)	94.2 ± 3.5	89.5 ± 3.5	<0.001	24
BMI (kg/m2)	34.0 ± 1.0	32.4 ± 1.0	<0.001	24
Waist circumference (cm)	110.5 ± 2.9	106.9 ± 2.9	<0.001	23
Hip circumference (cm)	118.8 ± 2.4	116.1 ± 3.0	0.057	23
Waist to hip ratio	0.93 ± 0.02	0.92 ± 0.02	0.041	23
Neck circumference (cm)	39.8 ± 0.9	39.0 ± 0.8	0.044	23

BMI, body mass index; BMD, Bone mineral density; n, sample size. All data are mean ± SEM. The significant difference was based on paired student t-test.

Glucose homeostasis

After 12 weeks of intermittent fasting, mean HbA1c for the combined groups ($n = 24$) reduced by 2 mmol/mol, from 43.5 ± 0.5 mmol/mol to 41.3 ± 0.4 mmol/mol ($p < 0.001$). There was no difference between the probiotic or placebo groups (**Table 4.2**), nor between males and females. HbA1c decreased in 21 out of the 24 participants (88 %) and 3 participants (12 %) achieved normoglycemia (HbA1c < 40 mmol/mol).

Anthropometric data

After 12 weeks of IF, both groups lost a similar amount of weight, ranging from 94.2 ± 3.5 kg to 89.5 ± 3.5 kg ($p < 0.001$, Table 4.2). There was no difference in weight loss between men and women. Across all completers, waist circumference and waist-to-hip ratio decreased ($p < 0.001$ and $p = 0.05$, respectively), and reductions were also seen for neck circumference, with a p-value of < 0.05. Notably, there were no significant differences between the two groups for these measures. Significant reductions in total body fat ($p < 0.001$), android fat ($p < 0.001$), and visceral fat ($p < 0.001$) were observed across all completers, with no difference observed between groups. For BMD, there was no clinical significant difference between the two visits with a p-value of greater than 0.05, and the same pattern was observed for hip circumference ($p > 0.05$) with no difference between participant allocations.

Effect of intermittent fasting on weight loss

Table 4.1. represents the mean ± SEM of body weight changes after 12-week intermittent fasting. For body weight, the mean values were 94.2 ± 3.5 kg before intervention, which changed to 89.5 ± 3.5 kg after the study concluded. For calculated BMI, a value of 34.0 ± 1.0 kg/m2 was observed and the mean for baseline intervention and this value was moved to 32.4 ± 1.0 kg/m2 for intervention data. All values were statistically significant with a p-value of less than 0.05. Furthermore, for both parameters, the percentage of changes were nearly the same with a value of -4.9 % changes in body weight and -4.7 % for changed BMI.

Table 4.3 Changes in body weight and calculated BMI after 12-week intermittent fasting

	Baseline visit	12 week visit	% changes	p value	n
Weight (kg)	94.2 ± 3.5	89.5 ± 3.5	-4.9	0.000	24
BMI (kg/m2)	34.0 ± 1.0	32.4 ± 1.0	-4.7	0.000	24

Fat changes after IF

Table 4.4 shows abdominal fat and pancreatic fat changes measured by MRI/MRS, including SCAT, VAT, LF and PF, before and after the 12-week dietary restriction, regardless of probiotic supplementation. Results from both PF quantification protocols are presented. Abdominal fat contents show highly significant reductions after the 12-week dietary intervention ($p < 0.001$).

Table 4.4 Fat changes after the 12-week intervention study

Variable	Baseline	12-week visit	p value
LF (%)	8.7 ± 0.8	7.5 ± 0.7	< 0.001
SAT (%)	35.9 ± 3.1	34.4 ± 3.2	< 0.001
VAT (%)	15.8 ± 1.3	14.8 ± 1.2	< 0.001
PF by Con. (%)	7.7 ± 0.5	6.5 ± 0.5	< 0.001
PF by MR-OPSY (%)	7.9 ± 0.6	6.3 ± 0.5	< 0.001
n	24	24	

BMI, body mass index; LF, liver fat; SAT, subcutaneous fat; VAT, visceral fat; PF, pancreatic fat; n, sample size. All data are mean ± SEM. The significant difference was based on paired sample t-test.

Liver Fat

Liver fat content decreased after weight loss in both groups after a 12 week period. At baseline, the mean LF value was 8.7 ± 0.8 % before intervention and then 7.5 ± 0.7 % after the study had finished, with a p-value of less than 0.001. The change in liver fat was similar for both groups.

Subcutaneous fat

At baseline, the mean LF value was 35.9 ± 3.1 % before intervention which changed to 34.4 ± 3.2 % after the study's conclusion, with a p-value of less than 0.001. The change in subcutaneous fat was similar for both groups.

Visceral fat

At baseline, the mean LF value was 15.8 ± 1.3 % before intervention and changed to 14.8 ± 1.2 % after the study had finished, with a p-value of less than 0.001. The change in visceral fat was similar for both groups.

Pancreas Fat

Pancreas fat did not differ significantly between the two allocation and quantification protocols. At baseline, a number of 7.7 ± 0.5 % was seen, eventually shifting to 6.5 ± 0.5 % ($p < 0.001$) after the 12-week intervention with conventional protocol use. For newly-adopted protocols like the MR-OPSY method, a number of 7.9 ± 0.6 % was seen, changing to 6.3 ± 0.5 % ($p < 0.001$) after the 12-week duration of this study.

PF Changes by protocols after 12 week of IF

Table 4.4 represents the mean ± SEM of pancreatic fat by using two different quantification protocols, namely conventional and MR-OPSY methods. For conventional protocol, the mean PF was 7.7 ± 0.5 % at baseline and 6.5 ± 1.5 % after the 12 week intervention. For the MR-OPSY method, the baseline PF mean was 7.7 ± 0.6, with the mean shifting to 6.2 ± 0.6 % at the second visit. However, when compared between the two visits, the effect of intermittent fasting yielded promising results as there were reductions in PF observed in the second visit with p value < 0.001 for both protocols. None of these pairs showed any statistical difference in statistical values ($p > 0.05$). To demonstrate the correlation between PF fat from two protocols, figure 4.1 is shown with BMI and PF from both methods.

Table 4.5 Comparison of pancreatic fat by Conventional and MR-OPSY methods after 12-week intervention study

	Conventional method	MR-OPSY	p value	Sample size
Baseline (%)	7.7 ± 0.5	7.9 ± 0.6	0.548	24
Follow-up (%)	6.5 ± 0.5	6.3 ± 0.5	0.259	23

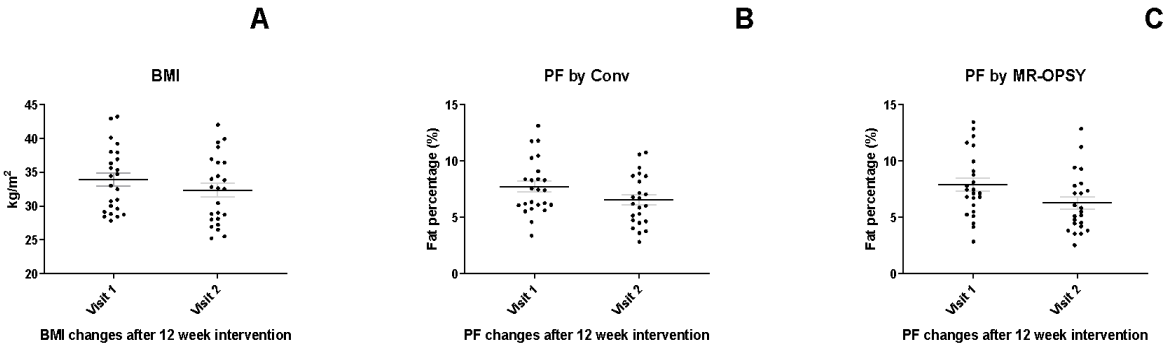

Figure 4.1 Comparison of BMI and PF changes after 12-week visit. A; Body Mass Index (BMI), B; Pancreatic fat by using conventional method (PF by Conv), C; Pancreatic fat by MR-OPSY method (PF by MR-OPSY).

PF method comparison by figures

Figure 4.2 shows figures of PF quantification by the conventional and MR-OPSY protocols. The figures represent ROIs for each method. In the conventional protocol, the whole pancreas was selected and used for PF quantification, while only three ROIs from the head, body and tails of the pancreas were selected in the MR-OPSY method. The figures also show an applied threshold for fat quantification that was used for PF measurements. All red pixels within selected ROIs were measured and calculated into PF fat percentage. These figures show that PF changes significantly after the 12-week study, with similar results seen in the calculated PF data.

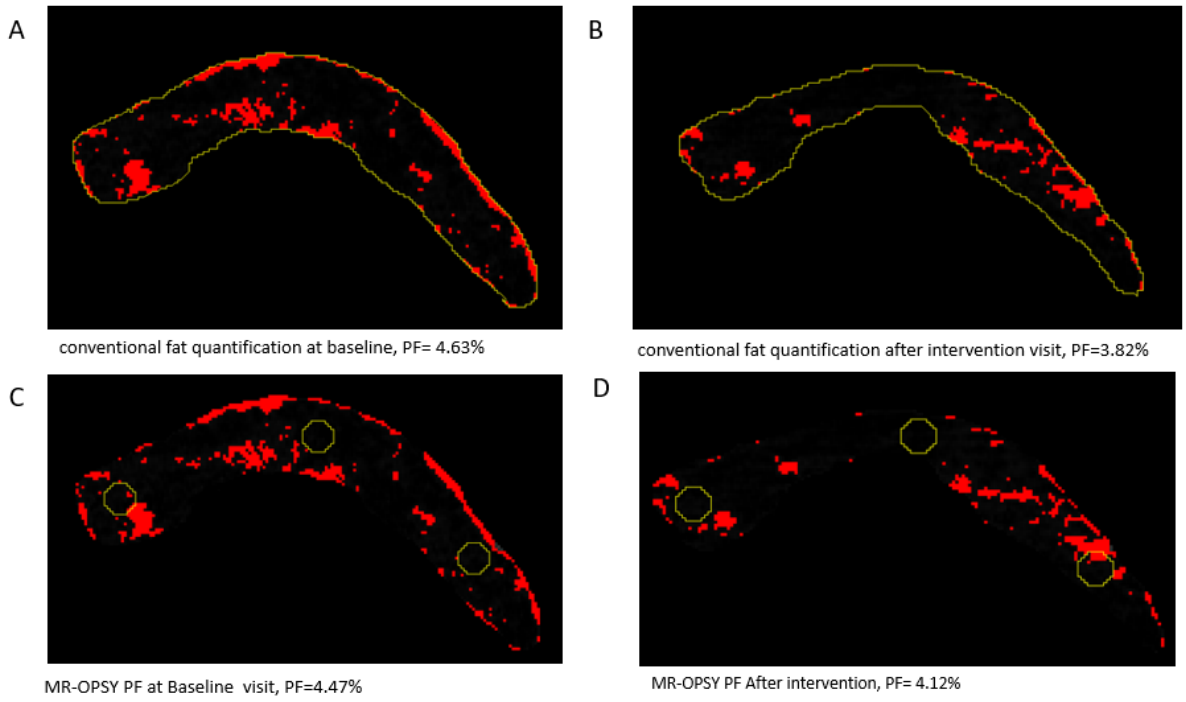

Figure 4.2 PF Comparison between two protocols after 12-week visits; A, baseline PF by using Conventional protocol; B, 12 week PF by using conventional protocol; C, baseline PF by using MR-OPSY protocol; D, 12 week PF by using MR-OPSY protocol

Figures 4.3 represents water and fat scans for calculating the pancreas volume. The water scan for individual participants at each visit was manually selected and extracted into a pure scan section. The whole pancreas area was then selected using the free-hand tool of ImageJ. All pancreatic slices were selected and used to calculate total pancreas volume. The slice thickness and numbers were used to yield the final pancreas volume. For pancreas fat volume, the same principle was used, but only fat pixels after thresholding were calculated and combined. The percentage of PF was then calculated by taking the ratio of PF versus PV, then multiplying by 100.

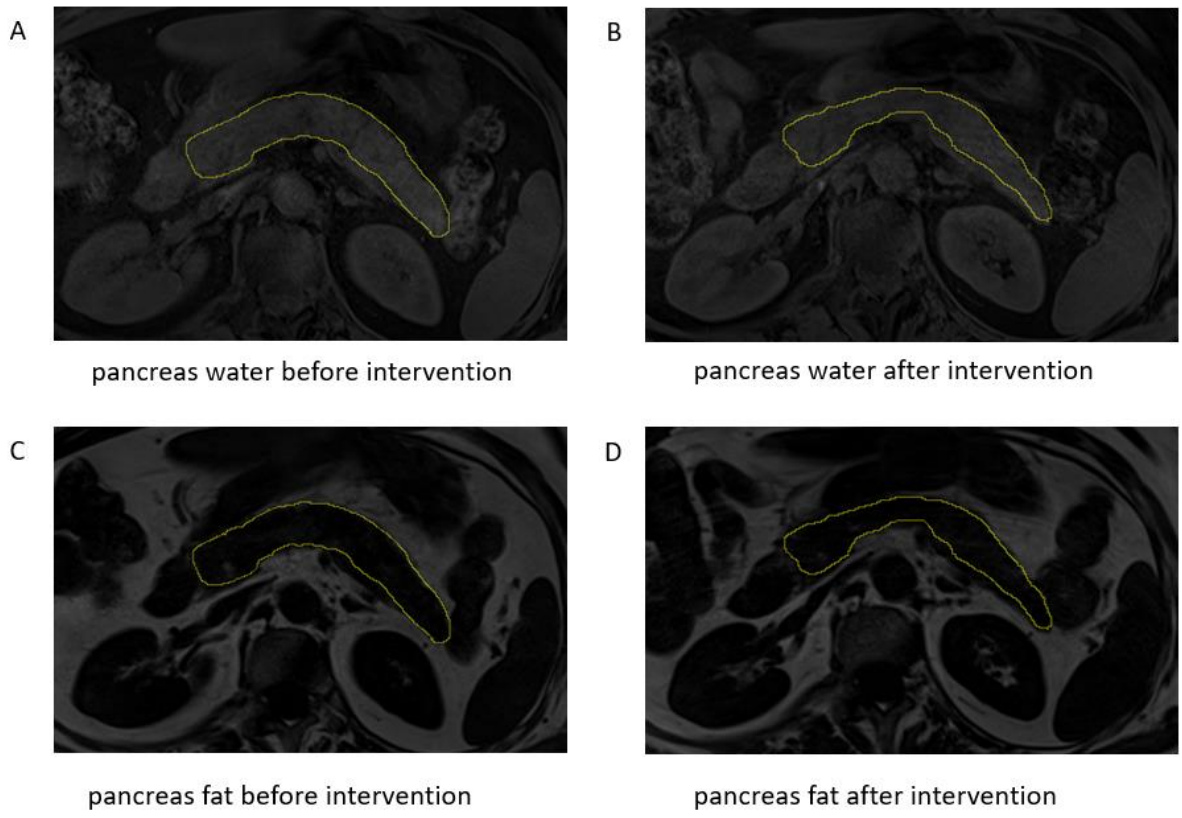

Figure 4.3 Pancreas volume after 12-week intervention taken from water and fat scans; A, pancreas water scan at baseline; B, pancreas water scan after 12 week intervention; C, pancreas fat scan at baseline; D, pancreas fat scan after 12 week intervention.

Method agreement between PF methods

Figure 4.4 shows correlation between the conventional and MR-OPSY protocol. Good correlation between the two was observed in both the percentage of PF and changes after the trial for PF data. A correlation of r = 0.924 was seen in the PF data, and a value of r = p.702 was present on PF changes after the study's 12-week duration.

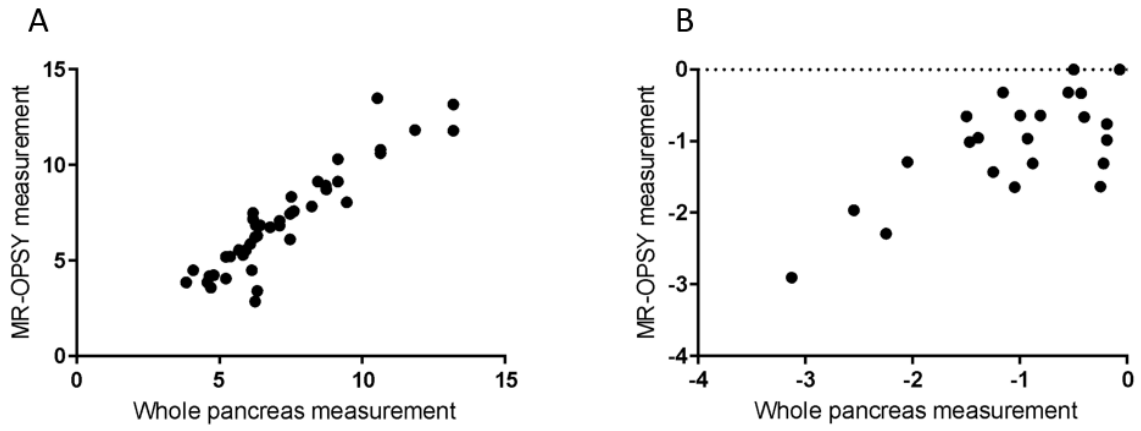

Figure 4.4 Pearson correlation between two PF quantification method; A, Pancreas fat volume, r=0.924; B, pancreas fat volume changes, r=0.702

Effect of Probiotics combined with IF

To investigate the effect of probiotic supplements, all parameter changes (i.e. the end of intervention measurement and baseline measurement) have been compared between the groups with and without probiotic supplement, as presented in Table 4.5 (anthropometric data, body composition and HbA1c) and Table 4 (abdominal fat). After the 12 weeks of this study, no significance could be observed between the two groups (with and without probiotic supplement) in any parameter (all $p > 0.05$).

Table 4.6 Comparison of parameter changes between two groups (probiotic supplement and placebo groups) after the 12-week intervention study

	IF (probiotic)n=10	IF (placebo) probiotic n=12	p value
Weight (kg)	-3.6 ± 1.2	-5.6 ± 0.8	0.154
BMI (kg/m2)	-1.2 ± 0.4	-1.9 ± 0.3	0.158
HbA1c	-2.0 ± 0.6	-2.5 ± 0.7	0.539
Total body fat (kg) (DXA)	-3.4 ± 1.0	-4.2 ± 0.8	0.456
BMD (G/CM2) (DXA)	-0.002 ± 0.005	-0.01 ± 0.006	0.228
Android fat	-0.5 ± 0.2	-0.6 ± 0.1	0.346
Visceral fat (kg) (DXA)	-0.2 ± 0.1	-0.3± 0.1	0.674
Waist circumference (cm)	-3.5 ± 1.9	-6.5 ± 0.9	0.314
Hip circumference (cm)	-0.8 ± 3.1	-4.7 ± 1.1	0.381
Waist hip ratio	-0.03 ± 0.02	-0.02 ± 0.01	0.497
Neck circumference (cm)	-1.0 ± 0.8	-1.3 ± 0.6	0.923

BMI, body mass index; BMD, bone mineral density; n, sample size. All data are mean ± SEM. The significant difference was based on the Mann-Whitney U test.

Glucose homeostasis

After 12 weeks of intermittent fasting, mean HbA1c for the combined groups ($n = 24$) reduced by 2 mmol/mol, from 43.4 ± 0.5 mmol/mol to 41.3 ± 0.4 mmol/mol ($p < 0.001$). No difference was observed between the probiotic and placebo groups (**Table 4.5**), nor between males and females. HbA1c amounts decreased in 21 out of the 24 participants (88 %) and 3 participants (12 %) achieved normoglycemia (HbA1c < 40 mmol/mol). In the IF group with probiotic supplementation, participants experienced a -2.0 ± 0.6 mmol/m of HbA1c while the number of -2.5 ± 0.7 mmol/mol was seen for the placebo group.

Anthropometric data

The mean ± SEM of body weight changes after the 12-week intermittent fasting by allocation is presented on Table 4.5. For body weight, the mean change was -3.6 ± 1.2 kg in the probiotic group, versus -5.6 ± 0.8 kg in the placebo group. Although the changes are seen as higher in the placebo group, no clinical statistical significance is seen, with a p-value of 0.154. For calculated BMI, a

value of -1.24 ± 0.4kg/m2 was observed in the probiotic group, versus -1.9 ± 0.3 kg/m2 in placebo data, with a calculated p-value of 0.158, indicating no statistical significance.

After the 12 weeks of IF both groups lost a similar amount of weight, from 94.2 ± 3.5 kg to 89.5 ± 3.5 kg ($p < 0.001$, **Table 4.5**). There was no difference in weight loss between men and women. Across all completers, waist circumference and waist-to-hip ratio decreased ($p < 0.001$ and $p = 0.05$, respectively). Reductions were observed in the neck circumference parameter, with a p-value of < 0.05. No significant differences between the two groups were observed for these measures. Significant reductions, however, were shown in total body fat ($p < 0.001$), android fat ($p < 0.001$), visceral fat ($p < 0.001$), across all completers, with no difference observed between groups. For BMD, there was no clinically significant difference between the two visits with p value of greater than 0.05 in which the same pattern was observed in hip circumference ($p > 0.05$) without any difference between allocations.

Fat changes after probiotic supplementation

Table 4.6 shows abdominal fat and pancreatic fat changes measured by MRI/MRS, including SCAT, VAT, LF and PF, before and after the 12-week dietary restriction by allocations. Results from both PF quantification protocols are presented.

Table 4.7 Comparison of BMI and fat changes between baseline and post-intervention(12-weeks)

	With probiotic	Placebo	p value
BMI (kg/m^2)	-1.2 ± 0.4	-1.3 ± 0.3	0.158
LF (%)	-1.2 ± 0.5	-1.4 ± 0.2	0.624
SAT (%)	-1.4 ± 0.4	-1.6 ± 0.6	0.733
VAT (%)	-1.3 ± 0.4	-0.7 ± 0.2	0.142
PF (%)	-0.9 ± 0.2	-1.4 ± 0.3	0.142
n	10	12	

BMI, body mass index; LF, liver fat; SAT, subcutaneous fat; VAT, visceral fat; PF, pancreatic fat by using conventional method; n, sample size. All data are mean ± SEM. The significant difference was based on unpaired sample t-test.

Liver Fat

Liver fat content decreased after weight loss in both groups after the 12-week period. At baseline, the mean LF value was 8.7 ± 0.8% before intervention and turned to 7.5 ± 0.7 % after the study had finished with a p-value of less than 0.001. In the probiotic group, a change of -1.2 ± 0.5% in LF was seen while a number of -1.4 ± 0.20 was shown in the control group with a p-value of 0.625, indicating non-statistical significance.

Subcutaneous fat

At baseline, the mean SCAT value was 35.9 ± 3.1% before intervention and turned to 34.4 ± 3.2% after the study had finished with a p-value of less than 0.001. In the study group, a change of -1.4 ± 0.4 % was represented while the number of -1.6 ± 0.6 % was shown in the placebo group with a p-value of 0.733, indicating no difference in changes of SCAT values between the two allocations.

Visceral fat

At baseline, the mean VAT value was 15.8 ± 1.3 % before intervention and turned to 14.8 ± 1.2 % after the study had finished with a p-value of less than 0.001. In the study group, a change of -1.3 ± 0.4 % was represented while the number of -0.7 ± 0.2 % was shown in the placebo group with a p-value of 0.142, indicating no difference in changes of VAT values between the two allocations.

Pancreas Fat

Pancreas fat did not differ significantly between the two allocation and quantification protocols. In the study group, a change of -0.9 ± 0.2 % was represented while the number of -1.4 ± 0.3 % was shown in the placebo group with a p-value of 0.733, indicating no difference in changes of PF between the two allocations. The data was acquired by using conventional PF protocols.

CHAPTER FIVE

DISCUSSION

Clinical characteristics

This is the double-blinded, randomized control trial study investigating the effects of probiotic supplementation on top of intermittent fasting, in which fat contents (namely subcutaneous, visceral, and liver fat) and HbA1c markers were assessed in obese pre-diabetic participants. HbA1c has shown to be a good indicator for estimating glucose homeostasis for up to 8 weeks beforehand, meaning glycaemic improvements could be assessed properly utilizing this marker.

Weight loss for PROFAST study

The 5 % weight loss achieved through intermittent fasting is close to the recommended 7% weight loss for diabetes prevention by the American Diabetes Association (1). This weight loss was achieved in 12 weeks and required little time investment from the study team, as follow-ups with participants were conducted via text message, phone call or email on a fortnightly basis. There was no additional weight loss or HbA1c reduction seen with probiotic usage, which highlights that clinically significant weight loss can occur with only two main components: dietary manipulation and regular virtual support. Unfortunately, there were insufficient resources to continue monitoring participants beyond the 12-week intervention, and thus we do not know whether weight loss and improvements in HbA1c were maintained once regular support was removed. We also cannot conclude that these results were due solely to intermittent fasting, as there was no comparison group using continuous energy restriction. Further research is also needed to establish whether this short-term intermittent fasting intervention can produce sustained weight loss and glycemic improvements.

As with all interventions, outcomes are dependent on participants' adherence to nutritional guidelines. Participants reported a moderate change between their baseline diet and their diet post-intervention, as well as an overall increased awareness of eating behavior. Although pretreatment binge eating disorder was not assessed, participants reported fewer episodes of overeating during

the intervention. This hints that adopting an intermittent fasting regime may have more positive than negative impacts on eating behavior. A systematic review published in 2015 found inconsistent associations between severe dietary energy restriction (i.e., low or very low energy diets) and the onset of binge eating in individuals without pretreatment binge eating(2). Therefore, this affirms that calorie restriction may not necessarily trigger adverse eating behaviors in those without binge eating disorder, however, pretreatment screening is still advised.

IF and probiotic supplementation for mental health

The PROFAST trial showed that daily supplementation of the *Lacticaseibacillus rhamnosus* HN001 probiotic in individuals with pre-diabetes who practiced intermittent fasting did not result in additional glycemic improvement or weight loss when compared with the placebo. The lack of significant differences between groups for weight loss and change in HbA1c suggests that the greater mental health improvements in the probiotic group were not attributed to these factors. Our results, therefore, offer new evidence that probiotics paired with intermittent fasting can improve mental health. Another New Zealand study investigated the effect of the same probiotic, *L. rhamnosus* HN001, versus a placebo on postnatal mood in women with gestational diabetes, finding that daily probiotic supplementation was associated with lower depression and anxiety scores in the postpartum period (3). Animal studies have also consistently shown that probiotics positively impact anxiety and depressive-like behaviors (4). Although obesity is associated with depressive and anxiety disorders (5), we did not compare our participants' scores with those of a normal New Zealand population. Further research is needed to explore the effects of probiotic supplementation and intermittent fasting among participants with depressive and anxiety disorders.

IF and probiotic supplementation in this study also adds to emerging evidence that intermittent fasting can reduce body weight and HbA1c. Weight loss in individuals with pre-diabetes, whether achieved via dietary or surgical methods, is consistently accompanied by reductions in HbA1c (6).

The magnitude of HbA1c reduction is often proportional to the baseline HbA1c, as greater improvements are achievable from higher HbA1c. In our study, eligible pre-diabetic participants had HbA1c levels of 40–50 mmol/mol, so the observed 2 mmol/mol reduction may appear modest when compared to other trials that recruited individuals who had T2D and thus a higher baseline HbA1c (7-9). Nevertheless, in view of other diabetes prevention trials, such a reduction in HbA1c represents considerable clinical success (10) and is comparable to results of weight management programs among people with pre-diabetes adhering to guideline recommendations (11). A systematic review and meta-analysis of 22 studies assessing T2D prevention in real-world settings suggests that modest reductions in weight (2.23 kg) can significantly reduce progression to T2D (11).

Continuous energy restriction and IF

Continuous energy restriction (CER) has long been studied as an approach for reducing weight (12) and improving metabolic complications. However, due to poor compliance and high drop-out rates (13), intermittent fasting (IF) has been conducted as an alternative to CER. For IF intervention study, participants were asked to restrict their energy intake to only 500-600 kcal per day, for 2 days per week. For the rest of the week, dietary consultation has been utilised for healthy eating, without eating beyond baseline requirements. In this study, we also concluded that IF yields a comparable result to previous studies (7, 14). Our results are in line with Varady et al.'s findings(7), in which after a 12-week duration subjects had a 4.9 % reduction in mean body weight after the intervention, compared to the 6 % reduction found in this study after the same time period. Harvie et al. (15) found that intermittent fasting was superior to CER in terms of insulin sensitivity and body fat reduction.

Body Fat contents Changes after IF in combination with probiotic supplementation

This study assessed fat changes in obese pre-diabetic participants after 12 weeks on intermittent fasting diet interventions, using the MRI technique for pancreatic fat content quantification as the primary outcome. Results showed positive outcomes for this intervention over the 12-week period

of the study in that both anthropometric parameters and PF reduced significantly. In this study, fat changes were assessed by changes in fat percentage instead of absolute volume of fat, because it is easier to compare the changes of fats for both visits and between quantification protocols. In this regard, the percentage changed would yield a clearer understanding rather than absolute fat volume.

Probiotics supplementations

Many have studied the effects of probiotics on fat metabolism and mechanism of actions (MOAs) of probiotics on fat metabolism. Mekkes et al. (16) proposed two MOAs, namely short chain fatty acid (SCFA) production and low-grade inflammation (LGI). These two MOAs could explain the influence of an obese gut microbiota inducing lipid storage and weight gain. They also proposed that probiotics might affect SCFA production by reducing the proportion of H^2-oxidizing microorganisms present in the microbiota. Probiotics can affect LGI by reducing the proportion of gram-negative microorganisms in the microbiota leading to a decreased level of Lipopoly-saccharide (LPS) in the bloodstream. As a result, LGI decreases and insulin sensitivity increases. Another randomized controlled trial (RCT) done in Finland where 256 pregnant women without metabolic disorders during their first trimester were recruited. They were divided into a dietary intervention group and a dietary with probiotics (*Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* Bb12) intervention group for the first trimester of pregnancy to six months after delivery. Both fasting blood glucose levels and glucose tolerance improved in the probiotics intervention group, and the likelihood of having gestational diabetes mellitus (GDM) declined significantly from 34 % to 13 %. Meanwhile, the probiotics-supplemented dietary group reduced the risk of central obesity in women at 6 months postpartum. These studies suggest that probiotic supplements may be good management tools for the prevention and treatment of obesity and diabetes (17-19). Although both studies yield a positive outcome, further studies need to be conducted to ensure the exact MOA behind probiotics and fat metabolism, especially in prediabetes participants.

Fat quantification

This study assessed fat changes in prediabetic participants after 12 weeks on an intermittent fasting diet, with and without probiotic supplementation. The results show that intermittent fasting can produce significant improvements in both anthropometric parameters and HbA1c. At the same time, the data do not show any significant beneficial effect of probiotic supplementation on top of intermittent fasting in terms of anthropometric parameters, body composition, abdominal fat and HbA1c.

Current evidence indicates that different parts of visceral fat have a different role on fat metabolism, namely intraperitoneal fat and retroperitoneal fat. As it is difficult to separate both stores of fats manually by MRI quantification, both portions were measured together as a single compartment. In addition, fat contents in bone marrow and intramuscular portions were excluded throughout the study. For subcutaneous fat, it is worth noting that metabolic consequences of superficial and deep subcutaneous fat (separated by a fascia) on metabolic syndrome in individuals may be different. However, the effects of these two parts remain unclear (20, 21), even though these two compartments could be assessed accurately by MRI (22). Therefore, it is decided to measure both portions as a single compartment in our study. The reliability of MRI techniques in comparison with gold standard methods like computer tomography (CT) scans has also been validated as a high correlation between the two (23).

MRS for Liver fat quantification

The MRS technique has been used to assess liver fat proportions in many studies (24, 25). The MRS protocol provides fast and reliable results in liver fat quantification. However, fat quantification by manual ROI selection has been identified as the most difficult way to get consistent results. In this regard, we validated the consistency of the quantification protocol by conducting blinded sample measurements. Previous studies showed reliable results by the utilization of MRS techniques for

accessing liver fat percentage (26, 27), and our liver fat data showed similar results compared with those studies. In addition, the SIVIC software has also been reviewed as a reliable software for liver fat quantification (28). The reliability of MRS when compared with liver biopsy has also been studied, and excellent correlation has been shown between the two methods (29). Due to the fast and non-invasive approach, MRS has also been used for mass-screening studies (24).

SAT and VAT by MRI

Many cross-sectional studies have demonstrated that VAT is a risk factor for metabolic syndromes (MS) in different ethnicities (30-34). However, the results of studies on the effect of SAT on MS have been inconsistent. Carr et al. (35) revealed that SAT is associated with MS (OR 2.12 per 1-SD of SAT), but they adjusted for only age and sex. Research from the Framingham Heart Study revealed that SAT was associated with MS after further adjustment for BMI, but they did not consider the effect of VAT (30). The Dallas Heart Study reported that SAT was not associated with MS after adjustment for VAT and BMI, while VAT was significantly associated with MS (36). However, SAT was inversely correlated with MS after adjusting for the SAT/VAT ratio(8). Our findings showed that the baseline SAT area was inversely associated with an increased risk of the individual components of MS (37). This association may provide a possible link regarding the protective effect of SAT on MS.

In many longitudinal studies, such as the MERLOT study, SAT was not associated with incident MS after adjustment for BMI, while VAT was significantly associated with incident MS even after adjustment for SAT (32). Similarly, the Framingham Offspring Study found that only VAT, not SAT, was associated with incident MS after adjustment for BMI and multiple risk factors (38). In accordance with these studies, our research revealed that only VAT, not SAT, increased the risk of incident MS when BMI, baseline fat tissue, and other traditional risk factors were considered. Regarding individual components of MS, both VAT and SAT were only positively associated with new onset hypertension, and showed no association with the incidence of diabetes, low HDL, or hypertriglyceridemia in the Framingham Offspring Study (38). Our study revealed that VAT was positively associated with the incidence of each component of MS, while SAT was inversely

associated with the incidence of high BP, high fasting glucose, and high TG, with marginal significance. While the criteria for hypertension and diabetes used in the Framingham Offspring Study were slightly different from our MS criteria for high BP and high fasting glucose, this difference in findings could be due to ethnic differences, and the results could indicate that SAT may be a 'metabolic sink' for metabolic abnormalities in Asians.

VAT is known to play a significant role in MS through various pathways (39). Ectopic VAT accumulation can cause dysfunctional alterations in adipose tissue, such as free fatty acid metabolism changes (40) and cellular hypoxia (41). Another possible mechanism is through adipokines; visceral obesity results in hypoadiponectinemia and an increase in tumor necrosis factor-α, interleukin (IL)-6, and other adipokines, which in turn result in insulin resistance (42). Additionally, it is well known that VAT secretes more proinflammatory molecules, such as complement C3 and tumor necrosis factor-α, than SAT (43). VAT also induces increased lipolysis and free fatty acids, which also causes insulin resistance (35, 44).

Both VAT and SAT secrete various proinflammatory molecules that could result in insulin resistance. Recent research has indicated that VAT is an 'ectopic fat' that originates from the 'overflow' of fat beyond the capacity of SAT to store extra energy. In this theory, when SAT reaches its limit to store extra energy, these excess TG molecules will accumulate at undesired sites, such as VAT (39). Therefore, peripheral SAT may exert a protective effect by decreasing fat deposition in the liver, muscle, heart, and VAT (39). The therapeutic effect of thiazolidinedione is also explained by the redistribution of fat from pathogenic VAT to less-pathogenic SAT (45). Recently, differential effects of deep and superficial SAT on metabolic risk factors have also been reported (46-48). The marginal effect of SAT on metabolic abnormalities in our study may be explained by the different effects of deep SAT and superficial SAT. Further studies are needed to better understand these relationships.

PF by MRI

Reproducible quantification of intra-parenchymal pancreas fat is important to allow comparisons between data from different research groups, and this is especially important as absolute

differences in pancreas fat between T2D and normal are modest (49-51). Previous studies demonstrate that higher inter-observer agreement can be achieved using MR-OPSY in comparison to the conventional region of interest method when intrapancreatic fat levels are higher, and pancreas volume is lower (52). As intrapancreatic fat increases and pancreas volume decreases with increasing disease duration (52, 53), the data are of particular relevance to this disease state. Re-analysis using the new method of previously published intervention studies of T2D, which used conventional methodology applied by experts, did not change the previously reported pathophysiological implications.

Several studies have demonstrated the association between increased intrapancreatic fat and T2D. In diabetes-prone rodent models of T2D, overfeeding brings about impairment of beta cell function, and this susceptibility to lipid availability is reflected in studies on isolated islets (54-58). In humans predisposed to develop T2D, prolonged intralipid infusion severely impairs beta-cell function (59). Conversely, removal of excess lipid from the environment of the pancreatic islet allows return of normal insulin secretion in early T2D (49, 51). This has also been observed in isolated islets (54). The apparent relationship of this lipid depot to the pathophysiology of T2D emphasizes the importance of a methodology for precise measurement.

Homogeneity of fat distribution within the pancreas is a topic of great debate (60-69). The series of studies on people with T2D showed a good degree of variability in pancreatic fat content between head, body and tail. Given that the biological relevance of this work is to investigate any effect of fat upon overall beta cell function and that these are distributed throughout the pancreas, inclusion of data from each region as a mean value to represent the whole pancreas is justified in order to represent fat distribution in the whole pancreas. Although selection of one region could be sufficient under certain conditions of homogeneous pancreas fat distribution, such as in a study of morbidly obese people, use of the MR-OPSY method is still a preferred option. A potential disadvantage of the method could arise if there was marked heterogeneity of fat content between regions of the pancreas, but the present observations and those of others suggest that this is rare. The pancreas in T2D diabetes is 30–50 % smaller than normal (52, 53), and the decrease in volume

as diabetes duration increases is accompanied by notable increase in irregularity in the pancreas borders. This implies greater likelihood of inclusion of the extra-pancreatic fat which exists between lobules (52). The contribution of pancreatic ducts or blood vessels which cannot be identified in the image has previously been overlooked. It is notable that the mean level of pancreas fat increased as a result of 1–20 % thresholding. The conventional method of pancreas fat quantification using magnetic resonance imaging has resulted in a wide range of reported pancreas fat content (51, 70, 71) and the present data suggest that this would be minimised by use of MR-OPSY. In previous studies the observers, who were experienced in image analysis but not in studying the pancreas, reported that placement of the 100 mm^2 MR-opsies was not challenging, and was also rapid (approximately 5 minutes vs. up to 30 minutes for conventional drawing round a region of interest) (72).

Short duration T2D can be reversed after weight loss with restoration of normal beta cell function, which is reported to be associated with a fall in intrapancreatic fat content (49-51). Application of the new method resulted in identification of no change in intrapancreatic fat in the longer duration group (which did not respond to weight loss by normalizing plasma glucose). These subjects had smaller, more irregular pancreases than the responders, participant who achieve normoglycemic level after the intervention period has reached, and the new method is more likely to reflect true intra-pancreatic fat levels. In the bariatric study, the T2D participants exhibited a notable return to normal glucose control (50), and quantitation of intrapancreatic fat by either conventional methods in expert hands or by the new method showed a significant decrease.

Several studies reported the robustness of MR-based fat quantification methods (73-75). A recent phantom study evaluated the reproducibility of MRI fat quantification technique between research centres, MR scanner vendors, field strengths, and acquisition protocols (76), emphasizing the importance of a standardized image analysis technique for precise comparison. However, the use of a phantom, an in-house auto thresholding software, does not reflect the complexities introduced by variable inclusion of visceral fat and fluid filled intra-organ ducts.

The published studies employing MR to quantify pancreas fat content used a wide range of methods for sampling size and location selected for fat quantification. This can partially explain the discrepancy in reported pancreas fat content. Of these studies, some reported a significant relationship between diabetes or insulin resistance and pancreas fat (50, 53, 60, 61, 77-82). Other studies reported no significant difference in pancreas fat content between type 2 diabetes and non-diabetic controls (51, 66, 70, 83-85). The remaining studies did not compare between non-diabetic and diabetic groups (50, 67, 71, 73, 86-94). When magnetic resonance imaging methods allow subsequent selection of the volume to analyse, magnetic resonance spectroscopy depends upon acquiring data from a volume of the body, pre-selected by imaging. Consequently, it is particularly susceptible to inclusion of visceral fat due to respiratory and other movement in the scanner, and such spectroscopy methods tend to report higher pancreas fat content (up to 24 %). Hu et al.(73) reported that MR spectroscopy was less accurate than imaging for pancreas fat quantification due to the difficulty in voxel positioning, which is consistent with the present data on the effect of selection of regions of interest for fat quantification. By combining (a) a selection of several regions to represent tissues from the whole organ; (b) a restriction of size of the selected region to decrease contamination from visceral fat; and (c) applying thresholding to exclude contribution from non-parenchymal tissues, an improvement in inter-observer agreement is observed.

Advantages of MR-OPSY for PF quantification

In the conventional method, delineating of the entire pancreas is challenging for the observer to get a consistent result. Time-consumption and expert-dependence are among the top issues for observers in this method for usage in clinical studies. In T2DM studies, many reported a wider range of results in PF (26, 27, 77, 83) that led to controversial issues regarding PF results for T2DM cases (80). Attaining high consistency in PF results demands high levels of experience and a large amount of time when conducting the study in large scale research (49-51). Moreover, conventional methods could yield large variations between observers.

The border of the pancreas is often irregular, especially in diabetes where the level of irregularity has increased in relation to increments in PF content (52). Furthermore, it is worth noting that pancreatic volume may decrease up to 30 % in T2DM individuals. This was demonstrated by Al-Mrabeh et al.(52) in 2016, where pancreatic volume decrease could reach up to 50 % after 10 years of having diabetes (53). Contamination of VAT in conventional PF quantification may lead to an overestimation of PF; many research projects have reported varied results when using conventional PF quantification (51, 61, 71)

Since the variation in PF contents is of great concern in this line of research, Al-Mrabeh et al. (72) proposed and showed that the MR-OPSY method has an advantage in reducing these issues. By selecting only 3 ROIs on the pancreas' head, body and tail, the PF calculation could be obtained after proper thresholding histogram data. By avoiding major blood vessels and ducts, the MR-OPSY method yields high precision results that could lead to a comparison between researchers. Reproducible PF data is important when comparing between T2DM and normoglycaemic subjects, especially when the difference between the groups are modest (49-51).

Disadvantages of MR-OPSY for PF quantification

Many studies found that pancreatic fat is varied across pancreas (60-62, 67-69). This issue could lead to the difference in PF between selected ROIs when using the MR-OPSY method and especially when severe fat infiltration has occurred. This could limit the use of this method for fat quantification in pancreas. When this issue has happened, the conventional method in which the entire pancreas is utilised could be better for total pancreatic fat calculation.

By applying only small portions of ROIs of 100 mm^2 within pancreas, the MR-OPSY method shows promising results for use in mass-scale study. However, as small portions of tissue regions were utilised, the efficiency of detecting changes of PF over time is yet unknown, particularly when follow-up study is required. When progressions of disease states were considered, PF increased over time, while PV decreased during the progression of T2DM (52, 53). Although conventional procedures are highly time-consuming and require highly skilled personnel, using the

conventional method shows promise in terms of follow-up study because when the total organ was utilised there is greater efficiency in detecting substantial changes. Further research needs to be performed to establish the use of these methods in long term intervention research.

Another issue of using MR-OPSY protocol is the placement of ROIs within the pancreas. By avoiding major blood vessels and ducts in pancreas, the need for high-resolution images is crucial. Differentiation between parenchymal tissue and vascular structure by MRI scanning needs further development. One of the factors relating to resolution of pancreas in MRI is breath-hold duration even with sparse scanning and more faster acquisition time (95). As the whole pancreas is used, the conventional method could be better for PF quantification in the case of low resolution MRI scanning.

The MR-OPSY method showed fast and reliable results as described previously (72). This study has applied MR-OPSY protocol in comparison with well-adopted conventional methods by ImageJ software. However, in the case of inhomogeneity of fat distribution of pancreas, the MR-OPSY protocol could yield a significant difference in comparison to the conventional method. This is because the entire pancreas volume was used for calculating the fat percentage within the organ, and the fat percentage would be more reliable than the MR-OPSY protocol. In case of ample homogeneity of fat distribution, especially low pancreatic fat percentage, the MR-OPSY method shows a very good correlation with the conventional method. When considering the time for each measurement, there is no doubt that the MR-OPSY method would allow mass-screening studies to become more feasible. When the pancreas fat percentage is high, conventional protocols could be used along with MR-OPSY procedures to ensure the more reliable results in pancreatic fat quantification.

In conclusion, we have compared the use of MRI quantification protocols for accessing pancreatic fat percentage in pre-diabetic patients performing intermittent fasting for up to 12 weeks. We have also shown that fat changes can be measured by MRI techniques.

CHAPTER SIX

SUMMARY, GENERAL CONCLUSIONS, AND RECOMMENDATIONS FOR FUTURE RESEARCH

Summary

This study presents the protocol and data analysis plan for assessing fat changes along with biomarkers for pre-diabetes improvement (as with HbA1c) using a randomized, double-blinded clinical intervention in obese subjects. The interventions are intermittent fasting with and without probiotic supplementation for between-visits and between-group comparisons. The results from this study will be helpful for decision-making of probiotic supplementation and intermittent fasting. As the protocol showed the comparison data between the two fat content quantifications, proper protocol for fat content measurements will be used for decision-making.

Intermittent fasting could help obese pre-diabetic participants lower their risk of developing diabetes by reducing abdominal fat content, including liver and pancreatic fat. It also showed significant improvement in anthropometric measurement and body composition. Hence, the utilization of intermittent fasting should be an effective means to control obesity and onset of diabetes with or without probiotic supplementation for obese pre-diabetic patients. The role of probiotic supplements in obesity and diabetes prevention requires further investigation.

In this study, we showed that both conventional and MR-OPSY protocols could be utilized for assessing pancreatic fat contents in a follow-up study up to 12 weeks. It is also shown that reliable pancreatic fat content data could be obtained by MRI quantification protocol. The results from the IF intervention showed that intermittent fasting could be utilized as a tool to reduce risks for developing of T2D in obese pre-diabetic participants. For probiotic supplementation, further study is needed for a better conclusion whether this intervention could yield any further benefit on top of IF.

Conclusion

CER has long been studied as an approach for reducing weight and improving metabolic health. However, due to poor compliance and high drop-out rates (1), intermittent fasting has been seen as an alternative to CER. It is worth noting that intermittent fasting intervention studies yield a comparable outcome with CER.

The PROFAST randomized controlled trial demonstrated that the *Lacticaseibacillus rhamnosus* probiotic did not have any additional benefits to weight loss or diabetes prevention when combined with an intermittent fasting intervention. Our results, though, do suggest that intermittent fasting may result in weight loss and diabetes prevention, which should be studied further. The key finding of this study is that the probiotic *L. rhamnosus* further improves social functioning and mental health outcomes when used together with intermittent fasting among people with pre-diabetes. Therefore, this study presents an opportunity for future research to further explore the psychological benefits of probiotics in this context.

The MR-OPSY method showed fast and reliable results as described previously (2), and this study has applied this protocol in comparison with well-adopted conventional methods by ImageJ software. However, in the case of inhomogeneity of fat distribution of the pancreas, the MR-OPSY protocol could yield a significant difference in comparison to the conventional method. As the entire pancreatic volume was used for calculating the fat percentage within the organ, the fat percentage would be more reliable than the MR-OPSY protocol. In case of sample inhomogeneity of fat distribution, especially when low pancreatic fat percentage was observed, MR-OPSY showed a very good correlation with the conventional method. When considering the time taken for each measurement, there is no doubt that the MR-OPSY method would allow mass screening research to become more feasible. When the pancreas fat percentage is high, conventional protocol could be used along with MR-OPSY procedures to ensure the most reliable results in pancreatic fat quantification.

Study Limitations and Future Recommendations

The main limitations of this study include the small sample size, the lack of an intention-to-treat analysis, and the absence of a comparison group using continuous energy restriction. Consequently, we are unable to recommend intermittent fasting over the traditional method of continuous energy restriction in clinical settings. Nevertheless, our results suggest that intermittent fasting may result in weight loss and diabetes prevention, which should be studied further. The results of the secondary analyses need to be interpreted cautiously owing to the multiplicity of tests conducted. Controlling for false discovery rate using Benjamini–Hochberg procedures (3) yields the smallest p-value of 0.392 (instead of 0.007) for mental health.

Several factors were not controlled for in this study, which may have influenced results. First, participants were instructed to maintain their habitual exercise patterns throughout the intervention period, however, this was not monitored to ensure compliance. If participants did alter their exercise levels, our observed results might not be due to intermittent fasting alone. Future studies could perhaps include specific exercise guidance and monitoring, which may also limit the reduction in fat-free mass and resting energy expenditure observed in the present study.

Given the emphasis on restricting calories ascribed to this intervention, the validity and accuracy of using self-reported food diaries to measure caloric intake are limited. Participants may have underreported caloric intake according to social desirability bias (4) or reactively changed their eating behavior to appear more adherent to the PROFAST protocol. Emerging evidence also suggests that dietary fiber can improve the functionality of probiotics, owing to a symbiotic approach (5). Although participants were encouraged to increase their fiber intake to increase satiety on fasting days, this was not adequately controlled for in our study and may have influenced our results.

The small sample number of participants who had finished the study is also considered a limitation of this study. Although with a proper blinded study and randomized design, this study could be

useful to demonstrate the usefulness of probiotics on top of intermittent fasting. The research team recruited the matched-control groups in which the other parameter apart from study interventions between the groups were minimized. In this study, ethnicity, age, and gender were recorded and examined for any confounding values that could lead to further study in the future

The number of participants for each group of interventions is one of the main limitations of this study. The absence of comparable groups with the same energy intake for continuous energy restriction, and the ratio of male and female participants could be considered another limitation, meaning that the interpretation of these results should be cautiously considered. Furthermore, the 12-week duration of this intervention study could not provide a more robust conclusion for the study outcomes. More participants and longer intervention times may allow for a better understanding of probiotics and fat reduction in participants. In addition, whether factors such as ethnicity have affected the outcome after probiotic interventions remain unclear. For fat measurements, reference automation software and expert advice would be helpful for establishing the correct measurement results.

The major limitation of the current MRI quantification study is the lack of a gold standard for non-invasive quantitation of fat solely within the parenchymal tissue of the pancreas. Presently, neither Dixon nor anatomical scans can differentiate between parenchymal tissue and ductal or small vascular structures in the pancreas. Optimization of image acquisition for differentiating between those small structures is demanding and currently being developed. For example, the T2-SPAIR sequence used in image 3 might allow exclusion of the main pancreatic duct within the MR-Opsy selection. As one of the major limiting factors for pancreas imaging resolution is breath-hold duration, the development of sparse scanning techniques which acquire data more rapidly may be expected to permit higher resolution imaging (89). Under the condition of severe pancreas fat infiltration of parenchymal tissues, the performance of the MR-OPSY method alone can be limited. Nonetheless, the proposed 20 % threshold to exclude areas of visceral fat invasion remains useful

under such circumstances, and values close to 20 % should trigger detailed examination of the pancreas' anatomy when selecting regions of interest. In conclusion, quantification of fat within the pancreas by MRI is significantly affected by the method of sampling, and the new MR-OPSY method allows higher inter-observer agreement. Application of this standardized new method with thresholding should permit measurement of changes in true intrapancreatic fat content which can reliably be compared between different research groups.

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Appendices

1. APPENDIX A

(Information sheet)

Participant Information Sheet

THE UNIVERSITY OF AUCKLAND
NEW ZEALAND
Te Whare Wānanga o Tāmaki Makaurau

Study title:	**PRObiotics and intermittent FASTing to improve prediabetes (PROFAST) study**		
Locality:	**Auckland**	Ethics committee ref.:	
Lead investigator:	**Dr Rinki Murphy**	Contact phone number:	**(09) 923 6313**

You are invited to take part in a study that aims to improve prediabetes (borderline high levels of glucose that signify high risk for developing type 2 diabetes) and overweight. Whether or not you take part is your choice. If you don't want to take part, you don't have to give a reason, and it won't affect the care you receive. If you do want to take part now, but change your mind later, you can pull out of the study at any time.

This **Participant Information Sheet** will help you decide if you would like to take part. It sets out why we are doing the study, what your participation would involve, what the benefits and risks to you might be, and what would happen after the study ends. We will go through this information with you and answer any questions you may have. You do not have to decide today whether or not you will participate in this study. Before you decide you may want to talk about the study with other people, such as family, whānau, friends, or healthcare providers. Feel free to do this.

If you agree to take part in this study, you will be asked to sign the **Consent Form** on the last page of this document. You will be given a copy of both the Participant Information Sheet and the Consent Form to keep.

This document is **7** pages long, including the Consent Form. Please make sure you have read and understood all the pages.

WHAT IS THE PURPOSE OF THE STUDY?

About the study

New Zealand people are getting heavier and as a result are suffering health problems, such as diabetes and heart disease. The good news is that by losing weight, people with prediabetes can reduce their risk of developing type 2 diabetes and benefit their present and future health.

Many bugs live in our gut and these bugs can influence our health. Probiotics (that is, bugs that are good for your health) may change the environment in the gut to improve how our body breaks down food and new research shows they can also help prevent diabetes and maintain healthy weight.

We hope to improve the health of people with prediabetes by undertaking a study called the **PRObiotics and intermittent FASTing for Prediabetes (PROFAST) Trial**. In this study, we will test two strategies:

1. Intermittent fasting: we will provide dietary advice on calorie counting in order to restrict calories to 600kcal per day for women or 650kcal per day for men for 2 days every week
2. Probiotic capsules (which are naturally occurring bugs with health benefits) may reduce high blood sugar and the chance of developing diabetes

The goals of the PROFAST study are:

1. To lower blood sugar and reverse prediabetes to normal levels
2. To lower body fat, particularly in the pancreas and liver
3. To investigate who responds best and whether we can predict this
4. To understand whether these strategies affect appetite, hormones and bacteria in the gut

The PROFAST trial is a four-armed randomised controlled trial. This means that one-quarter of participants will each be allocated to one of the following four groups by chance:

1. Probiotic capsule plus usual healthy diet
2. Probiotic capsule plus intermittent fasting
3. Placebo capsule plus intermittent fasting
4. Placebo capsule plus usual healthy diet

The probiotic capsules used in this study are safe to use. They were used in a previous study in New Zealand during pregnancy to prevent gestational diabetes and to babies to prevent eczema in children. The placebo (no probiotic) capsule looks the same as the probiotic capsule and allows the researchers to test the effect of the probiotics without being biased. While the study is in-progress, neither the people taking part in the study or the researchers assessing the people taking part in the study will know who is on probiotics or placebo. At the end of the study we will find out if probiotics and/or intermittent fasting normalises prediabetes and prevents diabetes.

PROFAST Feasibility study

Before we start the full study (560 people), we will do an initial **PROFAST Feasibility study** (44 people).

The goals of the initial **PROFAST feasibility and dose ranging study** are:

1. to assess the delivery and acceptability of the intermittent fasting intervention
2. to assess whether a lower dose of the probiotic produces similar benefits to the previously tested dose

The **PROFAST feasibility study** is a 2 stage, 2–armed randomised controlled trial. In the first stage, half of 22 participants will each be allocated to one of the following two groups by chance:

1. Probiotic capsule (standard dose) plus intermittent fasting
2. Placebo capsule plus intermittent fasting education

If analysis of these first stage results shows no difference, then we will use a higher dose of probiotic in the next 22 participants. Otherwise, if a benefit of the standard dose probiotic is seen, then in the second stage, half of the next 22 participants will each be allocated to one of the following two groups by chance:

1. Probiotic capsule (lower dose) plus intermittent fasting
2. Placebo capsule plus intermittent fasting

Who is doing this research?

The PROFAST trial is being done by researchers from: the University of Auckland; and Auckland University of Technology. The research team includes people working in the areas of diabetes, nutrition, body imaging and Pacific health.

If you have questions about the study, please contact:

Dr Rinki Murphy, lead researcher of the PROFAST trial

Phone: (09) 923 6313

Email: R.Murphy@auckland.ac.nz

Statement of Approval

This study has been approved by the Health and Disability Ethics Committees (HDEC), reference number XXX.

WHAT WILL MY PARTICIPATION IN THE STUDY INVOLVE?

Is this study suitable for me?

You are invited to take part in this study if you have prediabetes on your screening blood tests (HbA1c 41-49) and if you are overweight and above 18 years of age. Overweight is defined by a body mass index (BMI) of 30 or more (27 or more if you are of Asian Indian ethnicity). Body mass index (BMI) is a number calculated from a person's weight and height to provide an indicator of body size.

We intend to enrol 560 such people with prediabetes to take part in the **full PROFAST trial**, and 44 people with prediabetes to take part in the **initial PROFAST feasibility study**.

Are there any criteria for people to not take part in the study?

You will not be invited to take part in the study for the following reasons:

- Having previous or current diabetes
- Currently taking probiotic capsules or supplements containing probiotics
- Having had weight loss surgery
- Taking certain medications or having medical conditions that affect the breakdown of sugar in the body
- Planned major changes in physical activity during the study
- Weight change of >3kg within 3 months prior to first baseline visit
- Pregnant women or breastfeeding women or intending to become pregnant during the study duration
- Not accepting 5:2 intermittent fasting or daily probiotic supplementation
- Not confident with reading and writing in English
- For the MRI studies, you cannot take part if you have any implanted metal or electronic devices eg: metal implants, heart pacemakers, insulin pumps, implanted hearing aids, intracranial metal clips, metallic bodies in the eyes.

If I agree to take part, what will I be asked to do?

You will be asked to come to Auckland City Hospital, level 3, body composition unit for detailed assessments at baseline, after 12 weeks, and after 1 year:

Before each of these 3 visits (baseline, after 12 weeks and after 1 year):

- You will be asked to keep a **7-day food diary**. Please record everything you eat or drink as accurately as possible in the diary provided, or store a photo on your phone.
- You will be give a faecal sample collection pack to collect and store a **faecal (poo) sample** one or two days before your body composition unit visit.

  The types of bacteria present in your faecal sample will be analysed, along with short chain fatty acids they produce. It is important that the sample is frozen as soon as possible after collection to preserve all the bacteria in their original condition and prevent certain types of bacteria from overgrowing.

On the morning of the body composition unit visit, you will be asked to remember not to eat or drink anything other than water from midnight the night before for the following tests (which will take approximately 4 hours):

- Complete questionnaires about your health, lifestyle and well-being
- We will measure your blood pressure, weight and height, and waist circumference
- **Oral glucose tolerance test**: We will insert a small cannula (very fine plastic tube) into your vein, then withdraw blood before and after a glucose drink, at 30mins, 60mins, 90 mins, and 120min after the drink. You will only feel the slight discomfort of the first cannula, and all other blood samples will be taken from the same cannula. A total of 80mls of blood will be taken during this test (less than a quarter of what is collected in a single blood donation).
  - From your blood samples, we will measure levels of hormones that control blood glucose. It is important that you do not have anything to eat or drink on the morning of the OGTT, as this can interfere with how much hormones your gut produces.
- **DEXA scan:** This measures body composition (bone, fat, muscle). The scan takes about 10 minutes. You lie on an open bed and a scanning arm passes quickly over the top of you. You have to lie quietly without moving, but it is not an unpleasant measurement. The scanner releases 2 types of very low dose x-ray, which is less than one tenth of that of a chest X-ray and less than what you would get on a short international flight to Australia.
- **MRI scan:** This is a body scan that has been used in hospitals for many years. It uses a magnetic field and radio frequency pulse to obtain detailed images of your organs without the use of x-rays. It will identify the amount of fat stored in your liver, pancreas or muscle very accurately. You will be asked to lie down in a relaxed state, without movement if possible, within the machine whilst it scans your abdomen. The scan will take around 30 minutes.

*From study entry for 1 year (or for 12 weeks in the **PROFAST feasibility study**)*

- *Daily (for all)*

All who agree to participate will be asked to take *one* probiotic or placebo capsule every day. You will not be able to know which one you are taking until the end of the study.

- *Routine dietary advice (for half of participants)*

People who are allocated to routine dietary advice will receive best practice information in a pamphlet produced by the NZ Ministry of Health. This contains dietary advice that follows current New Zealand guidelines for prediabetes.

- *Intermittent fasting (5:2) Dietary education (for half of participants)*

People who are allocated to the intermittent fasting dietary education groups will receive detailed advice on intermittent fasting. This will include 4 group sessions with a dietitian or dietitian-trained facilitator. The sessions will begin soon after joining the study and end by 12 weeks. Each group education session will last about 30 minutes to 1 hour. Written material will also be provided.

Will my GP be told I am in the study?

Yes, if you agree and provide us their details, we will also inform your GP.

If I need an interpreter, can one be provided?

Unfortunately, we do not have funding to provide interpreters, which is why people not confident in reading and writing English will not be able to participate

WHAT ARE THE POSSIBLE BENEFITS AND RISKS OF THIS STUDY?

What are the benefits of taking part?

Taking part in this research may improve your health. It may also help to plan a widespread programme to improve the health of other New Zealanders with prediabetes. There could be unexpected findings from MRI scan such as discovery of nodules, or tumours. If these abnormalities are found, we will inform you immediately and also your GP to ensure adequate follow up is in place. However, since the images are not routinely reviewed by a radiologist, we are unable to perform diagnostic scans for medical purposes of areas where you have known or suspected abnormalities. At the end of the main study, we will inform you of the overall results. Unfortunately, we are not able to supply you with the probiotic supplement free of charge after the study has ended, but if the intermittent fasting shows a beneficial effect, we will share our resources about this with you.

What are the side-effects of taking part?

The main side-effect is that we will need your time. We will provide you with a $40 petrol voucher at the end of the study as koha to thank you for your valuable contribution in participating.

Probiotics: There are no known major side effects of taking probiotic supplements used in this study.

Blood tests: There is some discomfort and the risk of bruising as a result of blood tests.

Scans: Low levels of radiation will be used for DEXA scan which is similar to the radiation exposure experienced in the cabin on a flight from Auckland to Wellington.

WHO PAYS FOR THE STUDY?

Funding to undertake this study is provided by Health Research Council of New Zealand. The probiotic and placebo pills are supplied free of charge to the researchers by Fonterra, a NZ company which specialises in making this probiotic preparation that is sold worldwide.

WHAT IF SOMETHING GOES WRONG?

If you were injured in this study, which is unlikely, you would be eligible **to apply** for compensation from ACC just as you would be if you were injured in an accident at work or at home. This does not mean that your claim will automatically be accepted. You will have to lodge a claim with ACC, which may take some time to assess. If your claim is accepted, you will receive funding to assist in your recovery.

If you have private health or life insurance, you may wish to check with your insurer that taking part in this study won't affect your cover.

WHAT ARE MY RIGHTS?

Do I have to take part?

Whether you decide to take part or not is entirely up to you. Your decision will not affect the medical care you receive in any way. If you agree to take part, you are free to withdraw at a later stage, without giving a reason.

Do I have to give blood and other samples to take part?

You do not have to give a blood sample or any other samples for this research if you don't want to. You also have legal rights over samples you give for research. You keep control over any sample you donate for research. The samples you give can only be used for ethically approved research related to obesity, diabetes and heart disease. If the lab finds something important from the tests that might affect your health, you will be told about it as soon as possible. If you agree on the Consent Form, we will also inform your GP.

We respect your cultural beliefs. You may hold beliefs about a sacred and shared value of all or any tissue samples removed. The cultural issues associated with storing your tissue should be discussed with your family/whanau as appropriate. There are a range of views held by Maori around these issues; some iwi disagere with storage of samples citing whakapapa and advise their people to consult prior to participation in research where this occurs. However, it is acknowledged that individuals have the right to choose. If you change your mind later, just let us know, and we will either return or destroy your sample and remove your data.

Can I find out the results of the study and whether I received the probiotic or placebo?

If you would like to know the results of the study and whether you received the probiotic or placebo, we will send you a summary of the study and your treatment allocation once the trial is complete.

What if new information becomes available?

Should new information become available during the project the researchers will discuss this with you and you can decide if you want to continue in the study.

Will my taking part in the study be kept confidential?

No one outside the small group of researchers doing the study will know any of your details. When the results of the study are published in a medical journal for other doctors and scientists to see, no information about individual people or family/whānau is used. If further information is required we may need to access your medical records. All of your medical information collected for the study stays strictly private. All information stored about you will have your name, address and other identifying details removed. Your identifying and contact information will be stored separately from all other information we collect from you. No one will be able to identify you from anything we record. All computers used will be password protected.

2. APPENDIX B

(Consent Form)

Consent Form

THE UNIVERSITY OF AUCKLAND
NEW ZEALAND
Te Whare Wānanga o Tāmaki Makaurau

Please tick to indicate you consent to the following:		
I have read and I understand the Participant Information Sheet.	Yes ☐	
I have been given sufficient time to consider whether or not to participate in this study.	Yes ☐	
I have had the opportunity to use a legal representative, whānau/ family support or a friend to help me ask questions and understand the study.	Yes ☐	
I am satisfied with the answers I have been given regarding the study and I have a copy of this consent form and information sheet.	Yes ☐	
I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time without this affecting my medical care.	Yes ☐	
I consent to the research staff collecting and processing my information, including information about my health.	Yes ☐	
If I decide to withdraw from the study, I agree that the information collected about me up to the point when I withdraw may continue to be processed.	Yes ☐	
I consent to my GP or current provider being informed about my participation in the study and of any significant abnormal results obtained during the study.	Yes ☐	No ☐
I understand that my participation in this study is confidential and that no material, which could identify me personally, will be used in any reports on this study.	Yes ☐	
I understand the compensation provisions in case of injury during the study.	Yes ☐	
I know who to contact if I have any questions about the study in general.	Yes ☐	
I understand my responsibilities as a study participant.	Yes ☐	
I wish to receive a summary of the results from the study.	Yes ☐	No ☐
I agree to provide fecal (poo) samples at study entry, 3 months and 12 months	Yes ☐	No ☐
I consent to a DEXA scan to measure body composition (overall bone, fat and muscle density) at baseline, 3 months and 1 year.	Yes ☐	No ☐
I consent to a MRI scan to measure pancreas and liver fat content, at baseline, 3 months, and 1 year.	Yes ☐	No ☐

DECLARATION BY PARTICIPANT:

I hereby consent to take part in this study.

Participant's name: _____

Signature: _____ **Date:** _____

DECLARATION BY MEMBER OF RESEARCH TEAM:

I have given a verbal explanation of the research project to the participant, and have answered the participant's questions about it. I believe that the participant understands the study and has given informed consent to participate.

Researcher's name: _____

Signature: _____ **Date:** _____

3. APPENDIX C

(SF-12 Health and well-being questionnaire)

Your Health and Well-Being

This questionnaire asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities. *Thank you for completing this questionnaire!*

For each of the following questions, please mark an ☒ in the one box that best describes your answer.

1. In general, would you say your health is:

Excellent	Very good	Good	Fair	Poor
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

2. The following questions are about activities you might do during a typical day. Does <u>your health now limit you</u> in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
a. <u>Moderate activities</u>, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	☐ 1	☐ 2	☐ 3
b. Climbing <u>several</u> flights of stairs	☐ 1	☐ 2	☐ 3

3. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a Accomplished less than you would like	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b Were limited in the kind of work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

4. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a Accomplished less than you would like	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b Did work or other activities less carefully than usual	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

5. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

Not at all	A little bit	Moderately	Quite a bit	Extremely
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

6. **These questions are about how you feel and how things have been with you <u>during the past 4 weeks</u>. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the <u>past 4 weeks</u>…**

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Have you felt calm and peaceful?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. Did you have a lot of energy?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. Have you felt downhearted and depressed?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

7. **During the <u>past 4 weeks</u>, how much of the time has your <u>physical health or emotional problems</u> interfered with your social activities (like visiting with friends, relatives, etc.)?**

All of the time	Most of the time	Some of the time	A little of the time	None of the time
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Thank you for completing these questions!

4. APPENDIX D

(MRI safety and consent form)

THE UNIVERSITY OF AUCKLAND
NEW ZEALAND
Te Whare Wānanga o Tāmaki Makaurau

uniservices

Centre for Advanced MRI

MRI SAFETY AND CONSENT FORM

Name _____ _____

Date of Birth ____/____/____ NHI _____

Weight _____ kg Height _____ cm

Magnetic Resonance Imaging involves the use of an extremely powerful magnet.
For your **safety** please answer the following questions

Have you had a previous MRI scan?	☐yes ☐no
Do you have **or** have you **ever** had a cardiac pacemaker?	☐yes ☐no
Do you have a brain aneurysm clip?	☐yes ☐no
Have you **ever** had an injury to the **eye** with a metallic object or fragment?	☐yes ☐no
Have you had any previous surgery?	☐yes ☐no
Please list _____	
Do you have any allergies to medications?	☐yes ☐no
Please list _____	
Do you have any of the following:	
Anaemia, blood disorders, kidney disease or seizures?	☐yes ☐no

FEMALE PATIENTS

Is there any chance that you could be pregnant?	☐yes ☐no
Are you currently breastfeeding?	☐yes ☐no

PLEASE ANSWER THE QUESTIONS ON THE BACK OF THIS SHEET

DO YOU HAVE ANY OF THE FOLLOWING?

Implanted cardiac defibrillator	☐yes	☐no
Implanted electronic or magnetic device	☐yes	☐no
Metallic stent, filter or coil	☐yes	☐no
Cochlear implant or other ear implant	☐yes	☐no
Heart valve prosthesis	☐yes	☐no
Any type of prosthesis (eye, limb etc)	☐yes	☐no
Joint replacement	☐yes	☐no
Screws, plates or wires in bones or joints	☐yes	☐no
Shunt (spinal, intraventricular, or heart)	☐yes	☐no
Vascular or drug access port or catheter	☐yes	☐no
Radiation seeds or implants	☐yes	☐no
Medication patches (Nicotine or hormone)	☐yes	☐no
Tattoo or permanent makeup	☐yes	☐no
Dentures or partial plate	☐yes	☐no
Hearing aid	☐yes	☐no
Shrapnel, bullets or other metal	☐yes	☐no

BEFORE ENTERING THE MR SCAN ROOM

You must remove all metallic objects, including jewellery, watches, keys, coins, credit cards, pens, cell phones, hearing aids, clothing with metallic zips and fasteners, metallic threads, or glitter finishes. You may be asked to change into a gown.

Owing to the loud noises emitted by the MR system, you will be given headphones or ear plugs to protect your hearing.

If you answer YES or are uncertain regarding any of the above, please contact us on (09) 303 5966 prior *to your appointment.*

USE OF YOUR IMAGES

As a University it may be useful to use your images (without your name or other identifying details) for all or some of the following purposes -

- education and training by Centre for Advanced MRI staff
- scientific publications, reports and presentations
- University teaching
- publicity material for the Centre for Advanced MRI
- the Centre for Advanced MRI website and websites of organisations we collaborate with (e.g. Siemens the manufacturer of the machine)
- publicity materials for non-profit organisations
- television documentaries or other public interest media
- databases that may be published on the internet

I give consent for my images to be used for the above purposes provided that all details that could allow me to be identified have been removed ☐**yes** ☐**no**

I confirm that the above information is correct to the best of my knowledge.

Signature _____ Date____/____/____

Screening form checked by _____

5. APPENDIX E

(Incidental finding policy)

THE UNIVERSITY OF AUCKLAND
NEW ZEALAND
Te Whare Wānanga o Tāmaki Makaurau

uniservices

Advanced MRI

Manual:	QUALITY MANUAL
Title:	Incidental Findings Procedure
Number:	A17.0
Version:	1.9

INCIDENTAL FINDINGS POLICY

Introduction

Research projects can produce unexpected incidental findings unrelated to the original purpose of the research but which may have significance for the study participants. These findings are usually made by staff or students who do not have the medical training and expertise to determine if the incidental finding is of significance or simply a scan artifact or recognized variant of normal. The management of these incidental findings including their detection, verification and notification is dealt with within the standard ethical application and approval process. Clear policies and procedures must be in place prior to the beginning of a research project to enable staff to deal with these findings should they occur.

This document is part of the Centre for Advanced MRI quality manual and sets out the standard way in which the Centre deals with this issue. This document has been reviewed and approved by the University of Auckland ethics committee and is provided to researchers as a guide to assist them with this issue within their own research projects. Researchers are not bound by this procedure. With the approval of an ethics Committee another procedure which maintains the highest ethical standards may be substituted, particularly where research has unique or specific aspects that need to be dealt with as special cases.

Normal Volunteers
Because images of normal volunteers acquired for technical or research purposes are not routinely reviewed by a radiologist, CAMRI does not perform scans of body areas where there are known abnormalities. Normal volunteers must read the Participant Information sheet before reading and signing a Volunteers Consent form.

Participant Information Sheet
This sheet must include statements along the following lines regarding the 'Detection of Abnormalities' -

> *"In the event that a condition which is assessed to be a clinical abnormality is detected through performing a scan on you, you will be informed of this and will be advised to consult your general practitioner or other health professional of your choice."*
>
> *Because the images are not routinely reviewed by a radiologist we are unable to perform diagnostic scans for medical purposes of areas where you have known abnormalities.*
>
> *You should be aware that once you have been informed that a clinical abnormality has been detected through perfoming a scan on you this could*

affect your ability to obtain insurance whether or not you take the matter further."

Participants declining to be informed of an incidental finding

The participant information sheet states that where a significant incidental finding is made, participants will be advised of this finding. If a participant does not wish to be advised of such findings then it is CAMRI's policy to exclude them from the research project. It is CAMRI's position that an informed decision not to receive information regarding an incidental finding is not possible due to the broad range of possible findings and consequences. Without knowing what may or may not be found, a participant is unable to determine in an informed way if they would wish to be informed of the finding.

Identifying a potential incidental finding

Researchers, radiographers and staff involved in the research could all possibly identify a '*potential*' incidental finding either during the scan process or during subsequent analysis of the data. It is the responsibility of the person who makes this potential finding to consult with their senior staff member (for example the Clinical Director of CAMRI) or the principal investigator (PI) of the study so the matter can be taken further. It is obvious that the detection of the potential incidental finding is usually made by researchers (including students) who do not have a medical training to determine their significance. Research scans at CAMRI are not routinely reviewed by a radiologist because this would require the appointment of a fulltime radiologist and significant additional funding.

The presence of a "potential" incidental finding, the person noticing it, its general description, the research scan number, and time and date of the scan should be written onto the patient consent form. All information relating to the expert radiology opinion we seek should also be written on the same form.

Following up on a potential incidental finding

In most instances the senior staff member or study PI will take responsibility for taking the potential incidental finding further. When the PI is unavailable, the responsibility will fall to the most senior staff member. In order to preserve confidentiality, it is important that the potential finding is discussed only with persons having the requisite skills and experience to determine the significance of the finding. Additionally images should not be reproduced, emailed or distributed in any way.

Determining the significance of a potential incidental finding

The next step is for the PI or senior staff member is to consult with an appropriately qualified medical practitioner to determine if the potential incidental finding is of true significance, is a variant of normal or artifact of the scan process. In many instances the Centre for Advanced MRI (CAMRI) can offer their assistance in this regard which will typically involve the viewing of the images by a specialist radiologist for a medical opinion. Because the images acquired by the research protocol will almost certainly not be adequate for standard diagnostic accuracy and further scanning is likely to be required, a diagnosis (or differential diagnosis) will not be offered. A simple *"Yes, the participant should be informed and further investigated"* or *"No further action is required"* will be provided. Additionally, no formal written report beyond this simple sentence will be offered. At the point of confirmation, the *potential* incidental finding becomes an *actual* incidental finding. It is emphasised that prior to this stage, there is no certainty that would allow a participant to be informed. If costs are incurred during the verification process these will be handed onto the research project.

Advising the participant of the incidental finding

If the finding is deemed to be of no medical significance then no information is communicated to the participant. If the findings are of medical significance then the PI or senior staff member, accompanied by a registered medical practitioner will take the responsibility for passing this information to the participant. CAMRI may be able to assist with this process.

Before disclosure, the name and date of birth of the participant must be verified between the person making the disclosure and the research participant.

Divulging information to the participant

The participant should be given a brief description of the finding (they may already have their images) including the area of the body and general nature, but without diagnostic and certainly without prognostic information. At disclosure, the patient consent form (with information about discovery and radiology opinion) must be made available. The participant should then be advised that it is strongly recommended that they follow this up with their general practitioner or health provider of their choice. If their general practitioner or other health provider wishes to make contact with the medical practitioner involved in the participant discussion, this can be arranged through CAMRI.

A CDROM containing copies of the images is offered to the participant with a general letter confirming the finding (see below). The participant should be advised to take the CDROM and letter with them to their medical provider when they visit. The PI or staff member **must** not discuss any possible diagnosis, treatment or prognosis with the participant. If the participant chooses not to follow the finding up with a health provider this is their choice.

If further MRI scanning is required, CAMRI will generally *not* undertake this. On rare occasions where the patient is referred back to CAMRI by an appropriate specialist, the scanning will be performed as a standard clinical case.

CAMRI will not provide a specialist referral as all further investigations and responsibility for the participants care would then rest with CAMRI. By providing a letter and offering to discuss this with the general practitioner or chosen health care provider, the participant can then determine the way in which he or she wishes to act on the advice offered.

Confidentiality

Only persons directly involved in discovering the potential incidental finding, confirming the significance of the finding and discussing the matter with the participant should have knowledge of the process or be involved. The Centre for Advanced MRI advisory board will be informed that an incidental finding has been made along with the general nature of the finding but no details which could identify the participant will be included. Strict confidentiality will be maintained and care should be given to ensure that no information is given out to others that could allow a participant to be directly or indirectly identified. All letters sent to research participants relating to incidental findings should be clearly marked "Private and Confidential" on the envelope. Importantly, images should not be distributed in any way.

Flowchart Summary

Participant informed of potential for incidental findings and procedure for managing these in the participant information sheet during consent

↓

Potential incidental finding by CAMRI staff, researcher or student

↓

Referred to principal investigator or senior staff member

↓

Expert opinion confirms if the finding is medically significant, a scan artifact or a variant of normal

↓

Participant informed by research representative and medical practitioner with referral to general practitioner or health care provider of their choice

↓

Copy of standard letter and CDROM of images given to the participant

Date
Name and Address of Participant

Re: Incidental MRI Finding

Dear *[Participants Name]*,

Thank you for attending the Centre for Advanced MRI recently as part of *[insert study name]*. As discussed with you, an incidental finding of [*brief description of finding including body area and general nature]* was identified on your MRI scan.

This letter serves to provide additional details of the procedure set out in our 'Participant Information Sheet' including suggestions for appropriate action from this point.

The procedure followed by the Centre for Advanced MRI in this situation has been approved by the University of Auckland ethics committee. In accordance with this, I would strongly recommend that you make an appointment with your general practitioner or preferred health care provider and take this letter with you to the consultation. I would be happy to discuss this finding with your general practitioner (or alternative) if they would like to telephone me at the number listed above.

Further detailed reporting of this finding was not possible because the images acquired during the study were not planned or performed for diagnostic purposes. This is because the study was undertaken for research or technical rather than diagnostic purposes and the images have not been formally reported by a radiologist. Unfortunately as a research institution, the Centre for Advanced MRI is unable to offer further diagnostic scanning. If further MRI investigations are required, these should be organised by your general practitioner (or alternative) or any specialists you may be referred to through the standard public and/or private medical facilities.

If you have not already received a copy of your images on CDROM and would like these, please contact our receptionist Marie Rooney on University extension 83366 and she will arrange for these to be provided to you.

The Centre for Advanced MRI maintains a strict confidentiality policy. The MRI Advisory Board will be informed that an incidental finding has been made along with the general nature of the finding, but no details which could identify you will be provided.

If I can be of any further help, or you have any questions that have not yet been answered, please do not hesitate to contact me.

Yours sincerely,

Operations Manager
Centre for Advanced MRI