High Value Nutrition – Priority Program

Peak Nutrition for Metabolic Health [PANaMAH]

Evaluating FEijoa foR DIabetes Prevention in a Multi-ethnic New ZeAlaND Cohort: the FERDINAND study

A COMMUNITY NUTRITION INTERVENTION IN INDIVIDUALS WITH PRE-DIABETES

Jennifer Miles-Chan[1], Rinki Murphy[1], Ivana Sequeira[1], Jia Jiet Lim[1], Mike Taylor[1], Wayne Young[2], Karl Fraser[2], Karin Schofield[3], Sally Poppitt[1]

University of Auckland[1], AgResearch[2], Intwood Consulting[3]
New Zealand

In collaboration with

Professor Garth Cooper, University of Auckland

A/Prof Lindsay Plank, Body Composition Unit, University of Auckland

Study protocol

PRINCIPAL INVESTIGATORS	ASSOCIATE INVESTIGATORS
A/PROF JENNIFER MILES-CHAN, PHD Director, Human Nutrition Unit; Senior Lecturer, School of Biological Sciences; University of Auckland Email: j.miles-chan@auckland.ac.nz	**PROF. SALLY POPPITT, PHD** Founding Director, Human Nutrition Unit; School of Biological Sciences, University of Auckland Email: s.poppitt@auckland.ac.nz
DR IVANA SEQUEIRA, PHD Senior Research Fellow, Human Nutrition Unit, School of Biological Sciences, University of Auckland Email: i.sequeira@auckland.ac.nz	**DR JIA JIET LIM, PHD** Research Fellow, Human Nutrition Unit, School of Biological Sciences, University of Auckland Email: jia.jiet.lim@auckland.ac.nz
PROF. RINKI MURPHY MBCHB, FRACP PHD Consultant Endocrinologist, Diabetes specialist; Associate Professor, Department of Medicine, University of Auckland & Auckland City Hospital Email: r.murphy@auckland.ac.nz	**PROF. MIKE TAYLOR, PHD** Head, Microbiome Laboratory; School of Biological Sciences, University of Auckland Email: mw.taylor@auckland.ac.nz
	DR SHAKEELA JAYASINGHE, PHD Research Assistant, Human Nutrition Unit, School of Biological Sciences, University of Auckland Email: sn.jayasinghe@auckland.ac.nz
	DR KARL FRASER, PHD Senior Scientist and Head, Metabolomics Laboratory; AgResearch, Palmerston North Email: karl.fraser@agresearch.co.nz
	DR WAYNE YOUNG, PHD Senior Scientist; Microbiome Laboratory, AgResearch, Palmerston North Email: wayne.young@agresearch.co.nz

PRINCIPAL INVESTIGATORS	ASSOCIATE INVESTIGATORS
	DR KARIN SCHOFIELD, PHD Intwood Consultants, Palmerston North Email: karin@intwood.co.nz
	MR KURT GRAYSON Research Assistant, Human Nutrition Unit School of Biological Sciences, University of Auckland Email: kurt.grayson@auckland.ac.nz
	MR WILLIAM ZHU Research Nurse, Human Nutrition Unit School of Biological Sciences, University of Auckland Email: william.zhu@auckland.ac.nz
	MR IBRAHIM MOHAMED Phd student, Human Nutrition Unit School of Biological Sciences, University of Auckland Email:ibrahim.mohamed1996@gmail.com
	MR JACK PENHALIGAN Phd student, Human Nutrition Unit School of Biological Sciences, University of Auckland Email: jpen110@aucklanduni.ac.nz
	MR KOK HONG LEIU Phd student, Human Nutrition Unit School of Biological Sciences, University of Auckland Email: klei603@aucklanduni.ac.nz

	MR SAIF FARAJ Phd student School of Biological Sciences, University of Auckland Email: sfar315@aucklanduni.ac.nz

COLLABORATORS	
PROF. GARTH COOPER MBCHB, DPHIL, FRACP, DSC Professor of Biochemistry and Clinical Biochemistry, University of Auckland, New Zealand Email: g.cooper@auckland.ac.nz	**DR KIEREN HOLLINGSWORTH, PHD** Head, Magnetic Resonance Imaging Translational and Clinical Research Institute Faculty of Medical Sciences, University of Newcastle upon Tyne United Kingdom Email:kieren.hollingsworth@newcastle.ac.uk
A/PROF. LINDSAY PLANK, PHD Head, Body Composition Unit Department of Surgery, University of Auckland Email: l.plank@auckland.ac.nz	**DR YUN SING KOH, PHD** Senior Lecturer, Machine Learning Cluster Department of Computer Science University of Auckland Email: y.koh@auckland.ac.nz
DR. OLIVIER GASSER, PHD Translational Immunology Group Leader, Malaghan Institute of Medical Research, Wellington, NZ Email: ogasser@malaghan.org.nz	**DR. DENISE CONROY, PHD** Senior Scientist, Stakeholder & Consumer Intelligence Team Leader, Plant and Food Research, Auckland, NZ Email: denise.conroy@plantandfood.co.nz
	DR. IVY GAN, PHD Scientist, Stakeholder & Consumer Intelligence Plant and Food Research, Auckland, NZ Email: ivy.gan@plantandfood.co.nz

Summary

The New Zealand National Science Challenge High Value Nutrition (NSC-HVN) programme aims to identify established and novel blood, urine and faecal biomarkers related to increased risk of type 2 diabetes (T2D); and in turn to undertake nutrition interventions that target these biomarkers and thereby decrease T2D progression in high risk individuals. Tranche 1 of the HVN Peak Nutrition for Metabolic Health (PANaMAH) Programme investigated susceptibility to T2D in 'at risk' individuals even at a low body mass index (BMI) and hence outwardly lean. This is likely due, at least in part, to deposition of storage fat in 'risky' central sites such as visceral adipose depots and the non-adipose ectopic sites of pancreas and liver. This has been termed the *thin-on-the-outside-fat-on-the-inside* or 'TOFI' profile.

Tranche 2 of the PANaMAH programme will investigate nutrition interventions to ameliorate T2D risk in carefully phenotyped lean and overweight individuals with pre-diabetes resident in New Zealand. Recent studies have shown that greatest success in improving metabolic health, including euglycaemia, can be achieved when long-term dietary intervention for improved glycaemic control is preceded by prior body weight and adipose mass loss achieved through the use of a low energy diet (LED) and subsequent suppression of weight re-gain (Astbury et al., 2018; Christensen et al., 2018; Lean et al., 2018). Identifying dietary components that can contribute to this long-term weight loss maintenance and hence improve glycaemic endpoints is key to this. In addition, evidence is building in support of personalised diets where glycaemic response to intervention may be predicted through integration of factors such as dietary intake, continuous glucose monitoring, serum metabolome and faecal microbiome (Korem et al., 2017; Thaiss et al., 2016; Valsesia et al., 2020; Zeevi et al., 2015). Previously the PANaMAH programme has focused on shorter-term dietary/food and beverage (F&B) intervention studies of up to 3 months, in small cohorts of up to 120 individuals, with no weight loss component and a 'one-size-fits-all' diet approach.

The FERDINAND Study builds on the previous PANaMAH programme, and significantly extends into a longer-term 6 month intervention in a larger multi-ethnic cohort of 'at risk' adults; with the aim of evaluating a F&B product that may contribute to improved glycaemia during both weight/adipose mass loss and longer-term weight loss maintenance. Plant-derived polyphenols, commonly found in fruits such as feijoa, may provide a novel nutrition approach with evidence from prior pre-clinical, and a human clinical study, demonstrating improvement in glycaemic parameters following short-term consumption of 150 mg/d commercially available whole feijoa powder. Use of machine-learning algorithms will integrate clinical responses and 'omics outputs to predict individual response to the intervention, and will include quantitative measurements of personalised postprandial glucose response (PPGR) to determine between-individual variability, and identify baseline parameters that predict this variability. The outcomes of PPGR from the FERDINAND Cohort, following 6 months consumption of feijoa whole fruit powder, will provide predictive algorithms that can be validated in a future follow-up assessment.

1. BACKGROUND

Type 2 diabetes - a global health concern

Worldwide diabetes and adverse metabolic health greatly contribute to the healthcare and economic burden, with over 90% of those diagnosed with diabetes classified as type 2 diabetes (T2D) (Zheng, Ley, & Hu, 2018). The prevalence of T2D has dramatically escalated from 110 million people reported in 1994, to > 350 million in 2013, and is predicted to further increase to > 600 million by 2040 (Ogurtsova et al., 2017). This metabolic disorder is responsible for the death of approximately 1.5 million people annually and is a major risk factor for cardiovascular disease (CVD), in turn killing 13 million people worldwide each year and accounting for ~25% of all-cause mortality (Lozano et al., 2012). New Zealand is no exception to these trends with the number of individuals with T2D having more than doubled in recent years, with >200,000 individuals with this metabolic disease (Beig, Khanolkar, & Cundy, 2018; MinistryofHealth., 2016). The rapid uptrend of T2D globally will lead to it being the 7th leading cause of death by 2030 (NCDRiskFactorCollaboration, 2017).

T2D is not only a health concern for Westernised nations such as Europe, North America and Oceania, but recent evidence shows increasing prevalence in developing Asian countries as well (Alberti & Zimmet, 2014); particularly China where T2D prevalence has been estimated to reach close to 70% by 2030 in comparison to 20% in Industrialised countries (Shaw, Sicree, & Zimmet, 2010).

Body weight, adipose deposits, ectopic organ fat

Weight gain (Ng et al., 2014) and an unhealthy diet and lifestyle (Temelkova-Kurktschiev & Stefanov, 2012) have been identified as the most significant risk factors for developing T2D. They lead to compromised energy homeostasis and lipo-regulation which may adversely promote the accumulation of fat in metabolically 'risky' deep subcutaneous and visceral compartments rather than 'safe' superficial subcutaneous adipose compartments (Chen et al., 2018; Tene et al., 2018). Increased visceral adiposity - particularly non-adipose ectopic deposition into key organs such as pancreas and liver - may alter normal physiological control and worsen insulin resistance (IR) and pancreatic β-cell dysfunction thereby worsening risk of T2D (Hocking, Samocha-Bonet, Milner, Greenfield, & Chisholm, 2013; Sattar & Gill, 2014).

Accordingly, ongoing strategies to manage T2D are aimed at diet and lifestyle changes. Several International T2D prevention trials (Christensen et al., 2018; DiabetesPreventionProgramResearchGroup, 2002; Li et al., 2008; Lindström et al., 2006; Ramachandran et al., 2006) have shown that implementing lifestyle interventions in high-risk individuals can prevent or at least delay the progression to T2D by 50% (Liu, Silvestre, & Poppitt, 2015). Diets characterised by both greater quantities and variety of plant-origin foods have been shown to improve glucose tolerance and decrease T2D risk (McMacken & Shah, 2017). While the precise mechanism by which they exert their beneficial effects are unknown (Kim, Quon, &

Kim, 2014), the ubiquitous polyphenolic phytochemicals contained within plants have been proposed to contribute to the delay in T2D progression (Dembinska-Kiec, Mykkänen, Kiec-Wilk, & Mykkänen, 2008).

Plant based whole fruit extract

Ubiquitous dietary phytochemicals or polyphenols, consumed as part of fruit and or their extract, have long since been recognised to have a role in ameliorating T2D risk (Xiao & Hogger, 2015). Numerous randomised control trials (RCTs) using plant-based dietary polyphenol rich products, consumed over 4 -12 week duration (Balzer et al., 2008; Bozzetto et al., 2015; Kar, Laight, Rooprai, Shaw, & Cummings, 2009; Mellor, Madden, Smith, Kilpatrick, & Atkin, 2013; Ochiai et al., 2014; Ogawa, Matsumae, Kataoka, Yazaki, & Yamaguchi, 2013; Rakvaag & Dragsted, 2016; Zibadi, Rohdewald, Park, & Watson, 2008), have been shown to significantly improve inflammation, glycaemia and oxidative stress associated with T2D.

Recent characterisation of New Zealand grown cultivars of feijoa (Apollo, Unique, Opal Star, and Wiki Tu) shows they are abundantly rich in phenolic compounds, particularly procyanidins B1 & B2, catechins and quercetin-3-galactoside (Y. Peng, Bishop, Zhang, Chen, & Quek, 2020). Benefits associated with consumption of feijoa (*Acca sellowiana* (O.Berg) Burret) have been attributed to its antimicrobial (Elfarnini, Abdel-hamid, Achir, Jamaleddine, & Blaghen, 2018; Smeriglio et al., 2019), antioxidant (Yaoyao Peng, Bishop, & Quek, 2019) and anti-inflammatory (Yaoyao Peng, Bishop, Ferguson, & Quek, 2018) properties. Feijoa whole fruit powder is commercially available in NZ as Feiolix® (https://feiolix.com/) under the Food Act 2014. The powder is rich in phenols and fibre (Cooney J. & Trower, 2015) as well as novel abscisic acid (ABA) (Zocchi et al., 2017) which is known to upregulate peroxisome proliferator-activated receptor (PPAR γ) to stimulate insulin secretion, glucose absorption and glucagon peptide like 1 (GLP-1) secretion (Bassaganya-Riera et al., 2011).

Administration of Feiolix® over a 16-week period in leptin deficient (ob-/ob-) mice significantly reduced weight gain, feed efficiency ratio, liver weight and cholesterol levels (Foo & Watson, 2015). In a RCT in 34 individuals with type 2 diabetes and mild to moderate hypertension the consumption of 150 mg/d, over a 12 week period, showed a significant decrease in blood pressure, fasting plasma glucose (FPG), glycosylated haemoglobin (HbA_{1c}), triglycerides (TAG), total cholesterol (TC) and low density lipoprotein (LDL-C)] in comparison to placebo (Taghavi, Hoseini, Panah, Masud, & Watson, 2012). The recommended daily dose is 1150 mg/d with high dose considered at 2300 mg/d calculated on the basis of total polyphenols by UV responsive molecules (UVRM) relative to the UVRM of 150 mg powder. The dose of 1150 mg/d was shown to be as efficacious in lowering blood glucose and TAG at 3 weeks, as the high dose, in a streptozotocin (STZ)-induced diabetes mouse model (unpublished data).

Glycaemic response to diet

The response to diet/dietary components, in particular the postprandial glucose response, remains poorly characterised in those at risk of T2D; despite postprandial response being clearly linked as an independent risk factor for development of T2D (AmericanDiabetesAssociation, 2015) and CVD (Gallwitz, 2009). There is growing evidence that glycaemic response of individuals to a dietary intervention is both highly variable and difficult to predict (Christinat, Valsesia, & Masoodi, 2020; Valsesia et al., 2020; Zeevi et al., 2015). Hence, predicting response and in turn personalising intervention diets to optimise glycaemic improvements in high risk individuals is a key next step in development of food and beverage products for amelioration of dysglycaemia and T2D.

Building on PANaMAH programme outcomes

In Tranche 1 our research team phenotyped a unique cohort of Chinese and Caucasian adults resident in Auckland, New Zealand in the *Thin on the Outside Fat on the Inside* (TOFI)_Asia study. In this study we observed a significantly worse profile of clinical risk biomarkers in the Chinese cohort when compared with a similar age and BMI-matched Caucasian cohort confirming that they were at worse risk and likely to develop T2D earlier than their Caucasian counterparts. Importantly, we also identified metabolomic biomarkers of prediabetes which may represent early biomarkers of disease, and noted the characteristic pattern of lipid 'overspill' into pancreas and liver in a sub-cohort of women who underwent magnetic resonance imaging (MRI) for pancreas fat and magnetic resonance spectroscopy(MRS) to identify liver fat.

Tranche 2 is investigating nutrition interventions to ameliorate T2D risk in carefully phenotyped lean and overweight individuals with pre-diabetes resident within New Zealand. Recent studies have shown that greatest success can be achieved when long-term dietary intervention for better glycaemic control is preceded by prior body weight and adipose mass loss. Identifying dietary components that can contribute to this long-term weight loss maintenance and hence improve glycaemic endpoints is key to this. In addition, evidence is building in support of personalised diets where glycaemic response to intervention (*personalised postprandial glucose response, PPGR*) may be predicted through integration of factors such as dietary intake, blood parameters including continuous glucose monitoring (CGM) and serum metabolome, faecal microbiome, and physical activity. (Zeevi et al., 2015) Our research program will further build on the PPGR algorithms proposed by Zeevi, Segal and colleagues, using a wider set of parameters including detailed body composition and ectopic organ fat and metabolomics data.

Systems nutrition and PPGR

Previously the PANaMAH programme has focused on shorter-term F&B interventions, in small cohorts of individuals, and interventions with no weight loss component and a 'one-size-fits-all' approach. The Evaluating FEijoa foR DIabetes Prevention in a Multi-ethnic New ZeAlaND Cohort (FERDINAND) study builds on the previous HVN programme, and significantly extends into a longer-term 6 month intervention in larger cohorts of at risk adults. The aim of the programme is to use a systems nutrition approach (demographics, anthropometry, clinical biomarkers and 'omics platforms-metabolomics, genomics/faecal microbiome; to predict personalised postprandial glucose response, PPGR) to evaluate whether commercially available feijoa whole fruit powder (Feiolix®) can contribute to improved glycaemia during both (i) short-term body weight loss, and (ii) longer-term weight loss maintenance.

2. STUDY OBJECTIVE

The objective of this study is to investigate the long-term effects of whole fruit feijoa powder, both during and following LED-driven weight loss on established and novel biomarkers of T2D, using an integrated systems nutrition approach; in a parallel design, RCT of 6 months duration, in a high risk multi-ethnic population with pre-diabetes resident in New Zealand.

Also, to develop a machine-learning algorithm that predicts PPGR in a sub-cohort of participants undergoing the longitudinal 6-month intervention; so that the predictive PPGR algorithm can be tested in a future study.

2. 1 STUDY AIMS

i. recruit a multi-ethnic cohort of overweight adults (BMI ≥ 26 kg/m^2) with pre-diabetes using American Diabetes Association (ADA) criteria for FPG (5.6-6.9 mmol/L), and characterise established blood biomarkers of T2D risk

ii. characterise body composition using body scanning techniques, incl. dual energy x-ray absorptiometry (DXA) for whole body and MRI/MRS (sub-group) for abdominal, pancreas and liver fat, to investigate the TOFI profile

iii. characterise PPGR, using CGM during oral glucose tolerance test (OGTT) and mixed meal tests (MMT); and associated demographic, anthropometric, clinical, metabolome and microbiome markers;

iv. assess response of 6-month dietary intervention on:

- glucose and related parameters, including FPG and postprandial plasma glucose (OGTT 2h glucose and MMT), using CGM
- HbA_{1c}, insulin, C-peptide, lipids
- body weight and body composition
- inflammatory and immune response
- plasma metabolomics
- faecal microbiome
- resting metabolic rate and glucose induced thermogenesis using indirect calorimetry (IC)

3. METHODS

3.1 TRIAL DESIGN

The trial will be a 2 arm, parallel design, double blind RCT. The dietary intervention will span 6 months, consisting of an initial acute 2-month weight-loss driven by LED (Phase 1) and followed by a longer-term 4-month weight loss maintenance (Phase 2).

The 2 intervention arms are as follows:

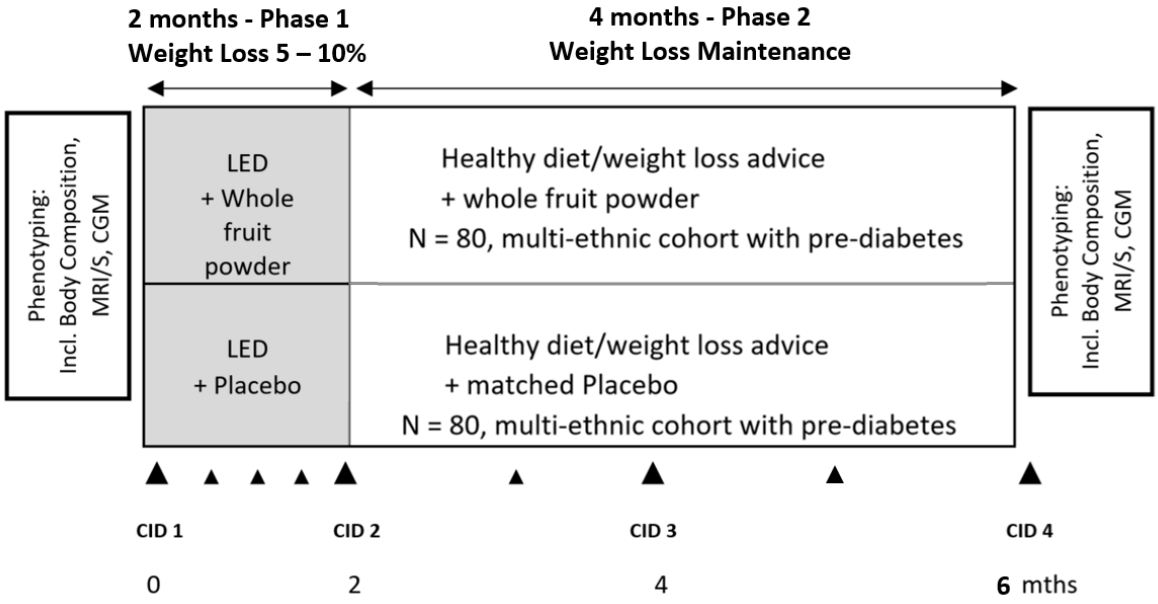
1. **Healthy diet/weight loss advice + feijoa whole fruit powder**

   Total meal replacement during the LED phase only (e.g. CID1-CID2), 4MJ/day; 1150 mg/d feijoa whole fruit powder daily in both phase 1 and phase 2 (to be consumed daily, with breakfast meal)

2. **Healthy diet/weight loss advice + matched Placebo**

   Total meal replacement during the LED phase only (e.g. CID1-CID2), 4MJ/day; 1150 mg/d matched placebo (e.g. bland non active commercial placebo like microcrystalline cellulose) daily in both phase 1 and phase 2 (to be consumed daily, with breakfast meal)

FIGURE 1. INTERVENTION ARMS



▲ Fading visit design; clinical investigation days (CIDs) and group diet advice (combination of in-clinic and virtual/online sessions); LED - wks 0, 2, 4, 6, 8 (5 time points); Maintenance - mths 3, 4, 5, 6 (4 time points)

LED, low energy diet (4MJ/day): total meal replacement provided as sachets (Cambridge Weight Plan^{TM} Ltd)

Body weight and composition (by dual energy x-ray absorptiometry, DXA) at CID 1/baseline, CID 2/end of LED, CID 3/4months & CID 4/end of study.

Magnetic resonance Imaging and Spectroscopy (MRI/MRS, sub-group, n=30; n= 15 placebo arm and n=15 whole fruit powder arm) assessment [7 day window] at CID 1/baseline, CID 2/end of LED, CID 4/end of study

Continuous glucose monitoring (CGM) over 4 days, for postprandial assessment incl. 2h OGTT and mixed (breakfast) meal tests; at CID 1/baseline $_{\text{all participants}}$, CID 2/end of LED (sub-group; n= 40 placebo arm and n=40 whole fruit powder arm), CID 4/end of study (sub-group; n= 40 placebo arm and n=40 whole fruit powder arm)

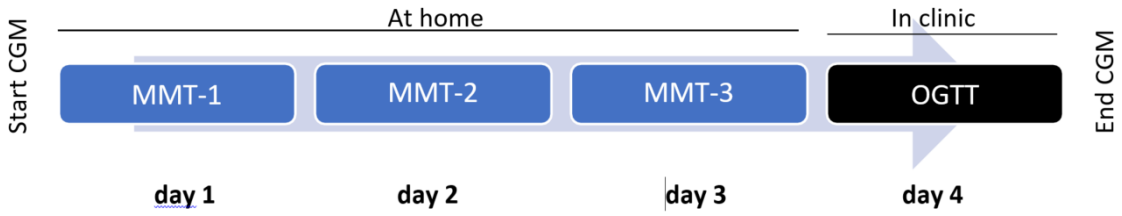
Indirect calorimetry (IC) in a subgroup of n = 30 (n = 15 placebo arm and n = 15 whole fruit powder arm) measured during 2h OGTT at CID 1/baseline, CID 2/end of LED, CID 4/end of study

CHARACTERISATION OF BASELINE PHENOTYPE – IDENTIFYING PREDICTORS OF PPGR

Demographic, anthropometric, clinical, metabolome and microbiome parameters will be integrated into an algorithm to predict PPGR, based on the methods developed in a cross sectional study of postprandial glucose control (Zeevi et al., 2015). Specifically, the PPGR for each participant will be predicted using a model developed from demographic (including ethnicity), anthropometric (including DXA, MRI and MRS), CGM including individualised postprandial OGTT and MMT responses, clinical, serum metabolome and faecal microbiome markers collected at the respective study visits.

CGMs are designed for self-insertion in home settings by individuals. For example they are routinely used by individuals with diabetes who need to monitor their blood glucose (https://www.mediray.co.nz/diabetes/shop/freestyle-libre-flash-glucose-monitoring-system/freestyle-libre-flash-glucose-monitoring-system). CGMs will be inserted under the skin; participants will be provided with the protocol and additionally guided/supported through the process on a video call, to allow continuous assessment of blood glucose levels throughout a 4-day period. If participants are uncomfortable doing this themselves then our study nurse will do this at the Human Nutrition Unit (HNU). Physical activity (PA) will also be assessed using a step count on mobile phone GPS technologies (e.g. Apple 'health' app) or simple pedometer if smart phone is not available. On Days 1-3 CGM assessments will be conducted at the participant's home and on Day 4 they will attend the HNU clinic for additional assessments (Figure 2).

FIGURE 2: 4-DAY PROTOCOL OF 3 MMTS CONSUMED AT HOME (1 PER DAY) AND AN ORAL GLUCOSE TOLERANCE TEST (OGTT) ON DAY 4 IN CLINIC.



MMT mixed meal test; OGTT, oral glucose tolerance test (plus IC, indirect calorimetry, in sub-group)
Physical Activity (PA), step count using mobile phone GPS technologies (eg. Apple 'Health' app)

On Day 1-3 of the home CGM assessment, a MMT will be conducted, which requires the participant to consume a single standardised breakfast meal at a fixed time on each of the 3 days. At the HNU clinic, on Day 4, an OGTT will be conducted (Figure 2) following the standard World Health Organisation (WHO) protocol with CGM and repeat venous blood sampling following cannulation and IC. CGM recordings will then be analysed to determine individual glycaemic response to the standardised meal.

The postprandial CGM assessments will be conducted prior to the start of the LED, i.e. 3 days prior to CID1/baseline visit in all participants; and similarly 3 days prior to CID4/8 months but only in the sub-group of participants randomised to the placebo and the feijoa whole fruit powder intervention arms (40 participants each arm; Table 1).

WEIGHT LOSS_LOW ENERGY DIET [LED]: PHASE 1

Commercial LED meal replacements (Cambridge Weight Plan™ Ltd) will be consumed for 8 weeks throughout Phase 1. LED is energy restricted with a target intake of ~4MJ/day. The target macronutrient composition of the diet will be 15-20% of total energy from fat, 35-40% from protein and 45-50% from carbohydrate. LED products include shakes, soups, bars, porridge, pasta and savoury meals.

- Participants randomised to the Healthy diet/weight loss advice + feijoa whole fruit powder arm will receive the LED products to be consumed as a total meal replacement with the whole fruit powder to be supplemented into the diet and consumed alongside the LED, with target EI of 4MJ/day.
- Participants randomised to Healthy diet/weight loss advice + matched Placebo arm will receive the LED products to be consumed as a total meal replacement with the matched placebo to be supplemented into the diet and consumed alongside the LED, with target EI of 4MJ/day.

During Phase 1 participants will attend, either in person or via virtual online mHealth platform, 5 group meetings delivered from the HNU clinic site. At each group meeting, adverse events (AE) and concomitant medications will be recorded; and dietary advice and behavioural instructions provided by dietitians. Participants will be recommended to maintain PA as per baseline (no increase) during the LED weight-loss phase. All participants will remain on the LED until their CID2 visit at week 8. It is expected that weight loss over 8 weeks will be between 5-10% of baseline body weight.

WEIGHT MAINTENANCE_DIETARY INTERVENTION: PHASE 2

During Phase 2 (4 months) participants will attend, either in person or via virtual mHealth platform, 4 group meetings delivered from the research site. The dietary advice will adhere to the New Zealand Ministry of Health healthy eating guidelines (McIntyre & Dutton, 2015) for improving metabolic health, with diet advice provided by dietitians.

CLINIC ASSESSMENTS

TABLE 1. SUMMARY OF CID ASSESSMENTS OVER THE 6 MONTH STUDY PERIOD

	Phase 1 - LED		Phase 2- Maintenance	
Assessments	CID1 M0	CID 2 M2	CID 3 M4	CID 4 M6
Demographics	x			
Height	x			
Body weight	x	x	x	x
Waist circumference	x	x	x	x
Blood pressure	x	x	x	x
Fasting blood sample	x	x	x	x
Concomitant medications	x	x	x	x
Adverse events	x	x	x	x
Questionnaires (e.g. QoL and *Bristol stool chart)	x *	X *	x	X *
DXA, body composition	x	x	x	x
MRI/MRS, pancreas & liver fat (subgroup, n = 30)	x (n=15 placebo + n=15 whole fruit powder arm)	x (n=15 placebo + n=15 whole fruit powder arm)		x (n=15 placebo + n=15 whole fruit powder arm)
Mixed meal tests (MMT) over 3 consecutive days, CGM; at home	x			x (subgroup; n=40 placebo + n=40 whole fruit powder intervention arm)
2-hr OGTT, CGM; in clinic	x	x (subgroup; n=40 placebo + n=40 whole fruit powder intervention arm)		x (subgroup; n=40 placebo + n=40 whole fruit powder intervention arm)
Physical activity/steps	x	x	x	x
Indirect calorimetry, IC (subgroup, n = 30)	x (subgroup; n=15 placebo + n=15 whole fruit powder intervention arm)	x (subgroup; n=15 placebo + n=15 whole fruit powder intervention arm)		x (subgroup; n=15 placebo + n=15 whole fruit powder intervention arm)
Diet diary, self-reported	x	x	x	x
24-h urine sample [N], diet compliance	x	x	x	x
Faecal sample collection	x	x		x

GROUP VISITS – a total of 9 group visits (Phase 1, LED: Week 0, 2, 4, 6, and 8; Phase 2, Maintenance: Month 3, 4, 5, and 6), combination of in person or via virtual mHealth platform will be conducted by dietitians throughout the intervention to provide dietary advice and support to maximise compliance and treatment success.

PARTICIPANTS

N = 80 per intervention group

Total sample size of 160 participants

INCLUSION

- Male and female
- Aged between 18 - 70 years
- BMI ≥ 26 kg/m^2 and body weight ≤ 150kg
- FPG in prediabetic range, 5.6 – 6.9 mmol/L
- Otherwise healthy, as per self-report
- Agreement to participate in a weight loss study

EXCLUSION

- Type 1 or type 2 diabetes mellitus
- Medications controlling glycaemia
- Current or history of significant disease including cardiovascular disease; pancreatic disease, or other digestive diseases including inflammatory bowel syndrome/disease, ulcerative colitis, Crohn's disease; cancer; plus associated medications including steroids (except topical steroids) and atypical antipsychotics in previous 3 months
- Recent body weight loss/gain > 10 % within previous 3 months or taking part in an active diet program; or current medications for weight loss; or intending to alter physical activity during following 12 months
- Previous bariatric surgery
- Smoker or vaper, current or in previous 6 months
- Recreational drug user, current or in previous 6 months
- Pregnant or breastfeeding women, current or in previous 6 months
- Dislike or unwilling to consume food items included in the study (including animal products), or hypersensitivities or allergies to these foods
- Unwilling/unable to comply with study protocol
- Participation in other clinical intervention study, current or in previous 6 months
- Considered unsuitable to participate by the PI

PARTICIPANT RECRUITMENT AND SCREENING

Recruitment will be conducted within Auckland, New Zealand. Those individuals interested in participating will be invited to contact the HNU clinic for written information on the study. Those participants involved in previous studies as part of the National Science Challenge High Value Nutrition PANaMAH program (HDEC ref: 16/STH/23, 17/NTA/144, 20/STH/51 and 21/STH/231), and who consented to being contacted for future studies, will also be invited to participate in this study.

Participant recruitment service will also be provided by MediActivate, a system which has access to patients' unidentified health data using the Conporto backend and is widely used in e-prescribing. MediActivate will send study invitations via emails and short messaging service (SMS) to patients who identified as potentially eligible for the study. Patients who are interested in the study are invited to contact researchers for more information and screening, i.e., contact with the researchers will be initiated by the prospective participants, with no health information or contact details provided to the researchers by either MediActivate or Conporto. To encourage patients to complete the online pre-screening questionnaire, we will donate $10 to the Red Cross Cyclone Relief Fund for each pre-screening questionnaire completed by a unique patient, specific to MediActivate's recruitment pathway. Completion of the online pre-screening questionnaire will provide the opportunity for researchers to contact the patient via telephone and discuss the study with them. Patients will be given as much time they require to consider their participation in the study. The Red Cross donation will be made irrespective of whether the patient chooses to participate in the study or not.

Data on gender, age, ethnicity, reported bodyweight and height, current medications, supplement intake will be collected via telephone/online pre-screening questionnaire to ensure that inclusion/exclusion criteria are met prior to attending the screening assessment in clinic. The self-reported information will be used to determine and calculate (using FINDRISC questionnaire, see Appendix) a predictive diabetes risk score. Were participants to have a moderate/high risk score (>12), the results will be discussed with the participant in person by senior investigators at the HNU during the in-clinic screen visit.

POWER CALCULATION

A *priori* modelling of sample size was conducted using change in FPG data obtained from the New Zealand arm of the PREVIEW study (Fogelholm et al., 2017), conducted in a cohort of overweight adults with prediabetes and of mixed ethnicity at the HNU, University of Auckland and similar cohorts in LED weight loss trials such as the

DROPLET (Astbury et al., 2018) and & DioGenes responder subgroup (Valsesia, Saris, Astrup, Hager, & Masoodi, 2016).

The sample size calculations are based on the assumption that the improvement in FPG achieved during 2 month LED weight loss (Phase 1) will be maintained between control and treatment groups during the following weight loss maintenance (Phase 2).

With a sample of 160 (80 per group) in a 2 arm, parallel design longitudinal study, baseline FPG 5.8 mmol/L (0.6 mmol/L SD), type I error $\alpha = 0.05$, power of 80%, and drop out of 20%, we can detect a between-treatment difference in FPG of 0.3 mmol/L. This is both a statistically and clinically significant difference. The estimates show that N = 64 individuals per group are required, which is increased to N = 80 to account for up to 20% drop out rate during follow-up over the 6 mth period.

CLINIC VISITS

The main assessment points will occur as follows:

- Screening
- Pre-intervention assessments
- CID 1 - Week 0/ baseline, start of LED weight-loss phase 1
- CID 2 – Month 2/ end of LED, start of weight-maintenance phase 2
- CID 3 – Month 4
- CID 4 – Month 6: End of Trial (EOT)

VISIT 1 - SCREENING VISIT

All participants will be fasted overnight prior to attending the screening visit at the HNU clinic, Mount Eden, Auckland. During the screening visit, a participant information sheet (PIS) will be provided to individuals and the study will be explained by the research staff. Written informed consent will be obtained from each of the participants. They will then be screened for eligibility. Demographics (age, gender, and ethnicity), medical history, current medication and supplement intake will be recorded. Anthropometry (height, body weight, waist and hip circumference, BMI and blood pressure) will be recorded. A fasting blood sample will be conducted to assess FPG.

If participants are eligible they will be enrolled into the study and randomised, using the online Research Electronic Data Capture (REDCap) secure web-based system, to one of the two intervention arms. Following this, they will then be scheduled to attend a pre-intervention assessment visit.

VISIT 2- PRE-INTERVENTION ASSESSMENTS ['HOMEWORK' PRIOR TO START OF CID1]

Prior to CID 1/baseline participants will complete a series of pre-intervention assessments, during a 2 week window. Written and verbal instruction, including video call, will be provided to participants for each assessment. These will include

- DXA, body composition scan
- MRI/MRS scan for pancreas and liver fat assessment
- collection of faecal sample for microbiome analysis
- 4 day weighed food record/24-h diet recall
- 3 day MMT assessment (CGM inserted 3 days prior to CID 1)
- 3 day PA assessment

- **(i) Body composition**

  Total and compartmental body fat, pancreas fat and liver fat content will be characterised using:

  Dual energy x-ray absorptiometry [DXA]

  DXA is based on the 3 component model of body composition, and uses 2 x-ray energies to measure body fat mass, lean mass, and bone mineral density. Scans will be conducted within the Clinical research Centre, Faculty of Medical and Health Sciences, University of Auckland (iDXA, software version 15, GE-Lunar, Madison, WI), using a standard imaging and body positioning protocol. The participant is required to lie recumbent on the open scanner bed for ~10 min. Body composition comprising total body fat, fat-free soft tissue and bone mineral content (BMC) as well as regional fat deposition will be determined from DXA whole-body and segmental scans. Whole-body scan images will be analysed for total fat mass (TFM), total lean mass (TLM), and fat-free mass (FFM = TLM + BMC). Total body fat percentage (%BF) will be calculated as TFM * 100/ (TFM + TLM + BMC). Abdominal fat mass (AbFM) will be determined from a region of interest (ROI) defined automatically with lower horizontal boundary placed at the top of the iliac crest and height set to 20% of the distance from this boundary to the base of the skull, with the lateral margins including the waist outline (Kaul et al., 2012).

  MRI – abdominal and pancreas fat

  MRI will be conducted at CAMRI to quantity abdominal and pancreas fat. Fast sagittal localizing abdominal images from diaphragm to pelvis will be acquired using 3D dual gradient-echo sequence (VIBE) 2-point Dixon method (Berglund, Ahlström, Johansson, & Kullberg, 2011). on a 3T Magnetom Skyra scanner (Siemens, Germany, VE 11A). VAT and SAT will be quantified from a single fat

fraction map at the L4-L5 intervertebral disc space (Schweitzer et al., 2015) using ImageJ (Schneider, Rasband, & Eliceiri, 2012). Pancreas fat will be determined using the MR-opsy method (Al-Mrabeh, Hollingsworth, Steven, Tiniakos, & Taylor, 2017) with thresholding (1 – 20 %) applied to eliminate any inclusion of non-parenchymal tissue.

MRS – liver fat

MRS will be conducted to calculate liver fat and performed using a respiratory-gated sequence (Bredella et al., 2010) with liver fat calculated, using the SIVIC software (Crane, Olson, & Nelson, 2013) from area under the curve (AUC) of water and fat peaks from non-water-suppressed spectra and presented as percentage volume/volume.

- **(ii) Faecal Microbiome**
  A single faecal subsample will be collected by participants using a well-established commercial collection kit. Participants will be given three home collection kits (OMNIgene•GUT, manufactured by DNA Genotek) in order to bring a faecal subsample to the HNU at CID 1, CID 2 and CID 4. Each OMNIgene•GUT microbiome collection kit includes: user instructions, spatula, a toilet 'accessory' to facilitate collection, biospecimen bag and a two way mailers shipping box. Participants will also receive the Bristol stool chart (see Appendix) with their faecal collection kit. Faecal samples collected by OMNIgene•GUT remain stable at room temperature for up to 60 days. Once received, samples will be transferred to -80^{o}C storage until analysis.

  Microbiome analyses will be carried out using a shotgun metagenomics approach (Quince et al., 2017). Firstly, microbial DNA will be extracted from each faecal sample using the Macherey-Nagel NucleoSpin DNA Stool Mini kit, followed by generation of metagenome libraries using Seqwell plexWell technology at Auckland Genomics Ltd. Library pools will be quality-checked using Illumina MiSeq then sequenced using Illumina NovaSeq 150PE. All sample preparation and sequencing will take place within New Zealand. Any sequence data resulting from remnant human DNA in the extract will be removed bioinformatically during the analysis process (described below). Additionally, regulation of the aryl hydrocarbon receptor (AhR), which is a ligand-activated transcription factor, which is expressed by a number of immune cells will be determined using a luciferase reporter assay method. AhR are reported to be regulated by small molecules in the diet and gut microbiome (Rothhammer & Quintana, 2019). However, the role of gut-microbiome-derived metabolites and diet on AhR signalling remains unclear.

Analysis of gut microbiome identity and function - detail

In line with standard practice, faecal samples will be used as a non-invasive proxy for the gastrointestinal microbiome. Because many bacteria have thus far resisted cultivation attempts, we will employ cultivation-independent, molecular approaches to characterise these microbial communities. DNA will be extracted from faecal samples and the identity and potential function of the bacteria present will be determined by best-practice approaches for characterising microbiomes (i.e. shotgun metagenomics based on read-based profiling). Sequencing will be undertaken by a commercial provider (Auckland Genomics Ltd) using next-generation sequencing technologies (Illumina NovaSeq). Bioinformatic and statistical analyses of the obtained sequence reads will be performed using read-based taxonomic and metabolic profiling. In brief, MEGAN software and the KEGG database will be utilised to provide novel data on functional potential across the microbiome dataset. Differential abundance of functional gene attributes will be analysed using DESeq2. The obtained sequence data will yield insights into the functional potential of the respective bacterial communities, allowing determination of, for example, whether certain groups of bacteria or bacterial genes are differentially represented in a specific study cohort (e.g. diet intervention). As per standard practice for microbiome/metagenome studies, microbial sequence data will be deposited (in a de-identified form) into a reference database at the time of publication in a peer-reviewed journal.

(iii) Dietary assessment using a 4-day food record

A 4-day food record, and/or 24-h diet recall, will be completed. In the 4-day record, food and beverages consumed will be recorded in a diet diary using a combination of weighed food portions and estimated portion size. The pan-HVN Tranche 2 overarching methods document (HighValueNutrition(HVN), 2018) recommends 24-h diet recall as the most accurate method for diet assessment during an RCT. Random within-person error can be mitigated by repeat measures and averaging. Variables known to be related to reporting accuracy (e.g. BMI) and to differential response bias (e.g. social desirability score) will be included as covariates. Weight of food consumed will be coded using the commercial dietary programme Foodworks (Xyris, Australia) and the energy, macronutrient [en% fat, carbohydrate (CHO), protein, and alcohol] and micronutrient profiles will be calculated.

(iv) Postprandial glucose response using CGM

Three 2-hr mixed meal tests (MMTs) will be conducted as described in Figure 2, to assess PPGR, using a CGM (the New Zealand Freestyle Libre System (Abbot, NZ) or similar). These are a lancet-free, single use units which typically comprise a Reader and Sensor, with ability to store data over 14 day intervals. The LibreView

software, or similar, will be used to analyse the data, and comprises a secure cloud-based system.

The CGM will be inserted prior to the start of the MMT and remain in situ throughout the assessment period (Figure 2). Three breakfast meals, one for each of the days, of fixed composition and each containing 50g available CHO will be used:

- Breakfast meal Day 1 (1MJ) – white bread, 50g available CHO
- Breakfast meal Day 2 (1MJ) – white rice, 50g available CHO
- Breakfast meal Day 3 (1MJ) – high fibre/β-glucan, e.g. oats, 50g CHO

The meal will be consumed at home at 0800h, over a 15 minute interval. All breakfast foods must be consumed in full, no other foods consumed, and participant to remain sedentary over the 2-hr test. Postprandial glucose from the interstitial fluid (ISF-G) will be recorded throughout the 2-hour test. The CGM will remain in situ and measurements recorded continually throughout the day.

(v) Physical activity (PA)

PA will be assessed through record of daily step count, using the built-in accelerometer, gyroscope and GPS functions on the participant's personal smartphone device. The accuracy of tracking step counts using smartphone devices has been validated against routine wearable devices (Case, Burwick, Volpp, & Patel, 2015). The Apple iphone 'Health' (https://www.apple.com/nz/ios/health/), Android 'Google Fit' app (https://www.google.com/fit/) or similar will be downloaded as free-ware. Participants will be requested to carry their smartphone throughout the 3-day MMT. Daily step count will be recorded, and data transferred to the HNU data hub when participants attend the HNU for their CID 1 visit. Alternatively, pedometers will be provided, by the researchers, to those participants without smartphone access.

VISIT 3 - CID 1/ WEEK 0/ BASELINE, START OF LED WEIGHT-LOSS (PHASE 1)

All participants will be fasted overnight prior to attending the study visit at the HNU. During the visit, the following will be conducted:

- Baseline assessments as outlined in Table 1. Including:
- 2-hr OGTT in all
- IC in subgroup; n=15 placebo arm & n=15 whole fruit powder arm
- Removal of CGM
- Provision of LED and dietary advice
- Schedule virtual group advice visits for weeks 2,4 and 6

(iii) Oral glucose tolerance test (OGTT) – in clinic

A 2-hr OGTT will be conducted in clinic to assess glycaemic response. Following review of the CGM device and insertion of an in dwelling venous cannula, participants will consume a 75 g glucose drink and both postprandial ISF-G and venous glucose will be recorded throughout the 2-hr test period. Standardised conditions for OGTT will be followed, including no food or beverage consumption and participants being sedentary. Venous blood samples will be collected at t = -5, 15, 30, 45, 60, 90 and 120 minutes for analysis of blood glucose and associated parameters including insulin and C-peptide. The CGM and cannula will be removed at the end of the OGTT.

Indirect calorimetry will also be conducted during the 2-hr test for a subgroup of participants.

Resting and postprandial energy expenditure - indirect calorimetry (IC)

Participants will be phenotyped in the fasted and postprandial state, in response to standardised 75g oral glucose drink (OGTT). Promotion of postprandial thermogenesis by diet is an important mechanism by which lipid oxidation as well as mobilisation of lipid stores from adipose and ectopic sites may be achieved. Furthermore, glucose/insulin-induced thermogenesis may serve as an early marker of progression towards, or amelioration of impaired glucose tolerance and T2D. Participants will be connected to equipment for cardiometabolic monitoring. Respiratory gas exchange will be measured non-invasively using an open-circuit ventilated hood system (Quark, Cosmed srl, Rome, Italy). Energy expenditure (EE) and respiratory quotient (RQ) will be calculated from the rates of oxygen consumption (VO_2) and carbon dioxide (VCO_2) production, e.g. resting metabolic rate (RMR) at 30 minutes and post prandial at 2-hrs. Heart rate will be measured by a wireless chest belt, and blood pressure by a digital sphygmomanometer.

Example of timeline for conduct of the OGTT + IC measures at CID 1

CID 1/Week 0/Baseline
7.30 am: bodyweight; cannulation + baseline blood sample (t = - 60)
7.45 am: body temperature & blood pressure, start IC (30-min RMR, fasted)
8.30 am: IC stopped (canopy removed), body temperature & blood pressure
8.40 am: Collect baseline blood sample (t = - 5)
8.42 - 8.45 am: Consume 75g OGTT glucose drink, t = 0 min, 2-h OGTT + IC (re-start)
9.00 - 10.30am: blood sample every 15 min, t = 15min to t = 90 min
10.45 am: final blood sample t = 120min, cannula removed, IC stopped (canopy removed), body temperature & blood pressure

VISIT 4- CID 2/ MTH 2, END OF LED, START OF WEIGHT-MAINTENANCE (PHASE 2)

2 month end of LED assessment as outlined in Table 1, detailed methodologies as previously described. Including:

- DXA, body composition scan
- MRI/MRS scan for pancreas and liver fat assessment
- 4 day weighed food record/24-h diet recall
- 3 day PA assessment
- 2-hr OGTT in subgroup; n=40 placebo arm & n=40 whole fruit powder arm
- IC in subgroup; n=15 placebo arm & n=15 whole fruit powder arm
- Transition from LED to healthy diet/weight loss advice
- Collection of faecal sample for microbiome analysis
- Schedule virtual group advice visit at mth 3

VISIT 5 - CID 3/ MTH 4, DURING WEIGHT-MAINTENANCE (PHASE 2)

Assessments as outlined in Table 1. Including:

- 4 day weighed food record/24-h diet recall
- 3 day PA assessment
- Schedule virtual group advice visits for mths 5, 6

VISIT 6 - END OF STUDY ASSESSMENTS ['HOMEWORK']

Prior to CID 4/mth 6 end of trial, participants will repeat the series of pre-intervention assessments, during a 2-week window. Written and verbal instruction, including video call, will be provided to participants for each assessment. These will include:

- DXA, body composition scan
- MRI/MRS scan pancreas and liver fat assessment
- collection of faecal sample for microbiome analysis
- 4 day weighed food record/24-h diet recall
- 3 day MMT assessment (CGM inserted 3 days prior to CID 4) only in the whole fruit powder group
- 3 day PA assessment

Visit 7 - CID 4/MTH 6, END OF TRIAL

All participants will be fasted overnight prior to attending the study visit at the HNU. During the visit, the following will be conducted:

- Assessments as outlined in Table 1. Including:
- 2-hr OGTT in subgroup; n=40 placebo arm & n=40 whole fruit powder arm
- IC in subgroup; n=15 placebo arm & n=15 whole fruit powder arm
- Removal of CGM

Compliance will be monitored over the intervention period (Table 1) from nitrogen balance, based on 24-hr urine collection. At in-person clinic visits (Visit 3, Visit 4 , Visit 5 and Visit 7) all participants will complete a quick 36 item short questionnaire (Table 1, QoL questionnaire; Appendix) to understand their views about their health, how they feel and how well they are able to do their usual activities. Scores for the different sections of the questionnaire i.e. a physical component summary and a mental component summary, will be tallied and pooled using a scoring key. The mental component only touches upon general mental health (psychological distress and well-being) and will be evaluated in comparison to the overall score. In the event that participants mental component score is significant to the overall score we will discuss this with our study clinician Dr. Rinki Murphy. Were she to consider this to be a significant result, she will inform participants and may contact their GP directly.

Additionally, thoughts and insights about the intervention will be collected during the study) during virtual online group sessions at week 6 (mid-way Phase 1, LED) and mth 4 (mid-way through Phase 2: weight maintenance).

This will involve an online survey and/or an online interview which will be conducted by the Plant and Food Research Team to understand participants' needs, wants and motivation in relation to diet, and health. The survey will be administered to all participants in the form of an anonymous questionnaire consisting of either 6 open-ended questions. All participants will be invited to take part in the survey and the focus group, but their participation is entirely voluntary. Participants may choose to take part in either the survey or the focus group, or both, and they may choose not to answer all questions in the survey, or during the focus group. The focus group interview will last ~1hr and conducted before/after the group session, when at the same time the survey will be administered in an online format to all participants for voluntary completion. Focus group conversations will be recorded, by the Researcher, to allow everyone's unique perspectives to be captured. Completed survey questionnaires will be kept anonymous by the Researchers. All information collected in the survey and focus group will be de-identified. *Any quotes which are used for reporting purposes will not contain details that could personally identify participants. De-identified conversations that are audio recorded, and later transcribed, as well as the*

anonymous questionnaires, will be kept securely by Dr Denise Conroy and Team at Plant & Food Research. NO names or other identifying information will be used, COLLECTIVE thoughts and ideas will ONLY be used for research purposes. Participants will receive a summary report of these interviews at the end of the study, if they choose.

DETAIL OF ADDITIONAL METHODOLOGIES

Clinical markers

Clinical blood and urine markers will be measured using standard biochemistry, ELISA, and similar laboratory techniques.

Immune profile

The ability to capture a snapshot of status of an individual's immune phenotype is vital to the understanding of how disorder develops, how NZ F&B products can mitigate the progressive loss of function, and as a monitor of overall health. Set against this are the confounding factors affecting human immunity -age, gender, general diet and lifestyle- which all have profound effects leading to broad variability in immune profiles between individuals. Consequently, to be most informative the immune profiling approaches for the High Value Nutrition programme cover several aspects of immune phenotype. This work is led by Dr. Olivier Gasser at the Malaghan Institute of Medical Research, Wellington.

Planned methods under investigation for the FERDINAND study include 1) flow cytometry-based technique which utilises a spectral analyser to allow detection of up to 48 individual characteristics in a single blood sample (which can be then deconvoluted to individual immune cell types such as T and B cells, monocytes), 2) a platform to assess the metabolic activity of cells *in vitro* in response to various stimuli, for instance metabolites associated with the intervention, and 3) high-throughput sequencing of the breadth and depth of unique B cell antigen receptors in a given sample (BCR-Seq). Together these techniques allow rapid in-depth immune profiling of participants and mechanistic insight into how dietary- and microbiota-derived metabolites impact immunity and homeostasis.

Analyses will follow the standardised HVN protocol; in brief, 1.5 mL of plasma (from ~5 mL of whole blood) will be collected at each CID, peripheral blood mononuclear cells (PBMCs) will be isolated using Lymphoprep™ (STEMCELL Technologies Inc.) system and stored at -80°C. Frozen samples will then be transported using dry ice and batch analysed at the Malaghan Institute of Medical Research, Wellington.

Metabolomics – Mass spectrometry

Metabolomics allows comprehensive high through-put measurement of a broad spectrum of metabolites with different chemical properties, utilising state of the art gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-high resolution mass spectrometry (LC-HR MS). The platform will primarily utilise a non-targeted mass spectrometry based (MS) approach to measure multiple metabolites from venous blood samples across a large dynamic range; with targeted GC-MS where required. Plasma (EDTA) will be collected and batch analysed at the end of the study.

Metabolomics analyses will be conducted at the Mass Spectrometry facility of AgResearch Ltd, Palmerston North (https://www.agresearch.co.nz/metabolomics).

A combination of multiple extraction solvents and analyses optimised for different metabolite polarity classes i.e. lipids, polar compounds such as amino acids, nucelotides etc., will be used. High resolution LCMS streams will be used for polar, semi-polar and non-polar metabolites, and GCMS for other polar metabolites not measurable by LCMS Identifications performed using in-house and external libraries, plus high resolution MS/MS to determine metabolite class, molecular formula for identification where required. Polar/semi-polar metabolites will be extracted from plasma and measured by LCMS using HILIC (hydrophilic interaction liquid chromatography) system coupled to high resolution Orbitrap MS detector; also TMS derivatisation and metabolites measured by GCMS; also LCMS using C18 (reverse phase) chromatography system coupled to a high resolution Orbitrap MS detector. Non-polar metabolites will be extracted from plasma and measured by LCMS using a CSH (modified reverse phase) chromatography system coupled to high resolution Orbitrap MS detector. In addition to high resolution detection of the molecular ion, this analytical system will collect fragmentation spectra of the major non-polar components to enable *in-silico* identification using the Thermo Lipid Search software package.

OUTCOME VARIABLES

Co-Primary outcomes

- fasting plasma glucose (FPG) at 6 months
- body weight at 6 months

Secondary outcomes

- $HbA_{1c,}$
- postprandial 2-h CGM-assessed ISF-G
    a. MMT
    b. OGTT
- body composition including:
    a. total body fat (DXA),
    b. abdominal body fat (MRI, sub-group)
    c. pancreas fat (MRI, sub-group)
    d. liver fat (MRS, sub-group)
- plasma metabolome profile
- indirect calorimetry (IC, sub-group) including:
    a. resting metabolic rate
    b. glucose-induced thermogenesis

Other outcomes

- clinical biomarkers including:
- OGTT venous glucose, fasting and OGTT insulin, C-peptide, lipid profile (total cholesterol, LDL-C, HDL-C, triglyceride), liver function tests, inflammatory & immune markers
- faecal microbiome
- urine Nitrogen (dietary compliance)
- epigenetic, SNP, miRNA markers of T2D
- PA assessment (step count)
- 24-hr glycaemic variability from CGM (e.g. evaluated using matrices such as standard deviation, SD; mean amplitude of glycaemic excursion, MAGE; mean of daily difference for inter-day variation, MODD; continuous overlapping net glycaemic action, CONGA)

BLOOD SAMPLES

Clinical parameters (glycaemic/metabolic health)

Metabolome

URINE SAMPLES

Nitrogen balance (compliance), 24-h collection

FAECAL SAMPLES

Microbiome + AhR activity

4. Ethics Approval

Human ethics approval to conduct this study will be obtained from the Auckland Health and Disabilities Committee (HDEC), Auckland, New Zealand.

5. Trial Registration

The trial will be registered with the Australia New Zealand Clinical Trials Registry (ANZCTR).

6. Risks and Benefits

Collection of blood samples is done by venous cannulation, which may result in mild discomfort for the participant. The participant will be monitored by a research nurse throughout the day and no adverse events are expected. Participants will be continuously monitored at all study visits and following the visits by telephone interview, over the study period, by the research staff.

DXA uses a low dose of ionizing radiation, similar to the natural radiation exposure of a 1-hr aeroplane flight, e.g. from Auckland to Wellington. The exposure to participants represents a very low risk. We do not foresee any safety concerns with regards to cumulative radiation exposure with this frequency of DXA scans. Natural background radiation is approximately 2.4 mSv a year or 6.7 μSv a day; a whole-body DEXA scan gives the equivalent radiation dose of 1 day or less (Bazzocchi, Ponti, Albisinni, Battista, & Guglielmi, 2016). Pregnancy in female participants is an exclusion criteria, as is metal implants such as cardiac pacemakers.

7. Data Collection/Privacy/Confidentiality

Data will be de-identified and recorded in hard copy on case report forms (CRF) and also stored in electronic format using Microsoft Excel. All hard copy CRFs will be stored in secure locked cabinets and the electronic data stored on a secure server with an automatic backup facility at the University of Auckland Human Nutrition Unit.

Data will be de-identified and recorded using the case report form (CRF) platform Redcap (https://projectredcap.org/), in addition to hard copy CRFs and also stored in electronic format using Microsoft Excel, with password protection. All hard copy CRFs will be stored in secure locked cabinets and the electronic data stored on a secure server with an automatic backup facility at the University of Auckland Human Nutrition Unit, AgResearch, Plant and Food Research and the Malaghan Institute.

8. Adverse Event Reporting

Adverse events (AEs) are classified as serious or non-serious. The investigator is responsible for reporting and recording adverse events. An adverse event is defined as an event that is undesirable occurring in a participant, whether related or unrelated to the study procedure.

Serious adverse events (SAEs) include:

- Death.
- Life threatening event.
- Serious injury i.e. events which require hospitalisation or medical attention.

Non serious events include:

- All events not defined as serious.

Any reported AEs and SAEs will be recorded throughout the 8 mth intervention period.

9. Data Retention

All data will be retained for a period of 10 years, or as stipulated by the NZ National Human Ethics Committee (HDEC).

10. Clinical Trial Sites

The study will be conducted at the Human Nutrition Unit, University of Auckland, with body composition assessments conducted at the Clinical Research Centre and the Centre for Advance MRI at the Faculty of Health and Medical Sciences, University of Auckland.

11. APPENDICES

APPENDIX: FINDRISC FORM FOR PRESCREEN ASSESSMENT OF PREDIABETES

Finnish Diabetes Association

TYPE 2 DIABETES RISK ASSESSMENT FORM

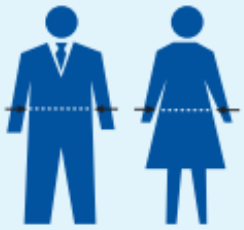
Circle the right alternative and add up your points.

1. Age
0 p. Under 45 years
2 p. 45–54 years
3 p. 55–64 years
4 p. Over 64 years

2. Body-mass index
(See reverse of form)
0 p. Lower than 25 kg/m²
1 p. 25–30 kg/m²
3 p. Higher than 30 kg/m²

3. Waist circumference measured below the ribs (usually at the level of the navel)

	MEN	WOMEN
0 p.	Less than 94 cm	Less than 80 cm
3 p.	94–102 cm	80–88 cm
4 p.	More than 102 cm	More than 88 cm



4. Do you usually have daily at least 30 minutes of physical activity at work and/or during leisure time (including normal daily activity)?
0 p. Yes
2 p. No

5. How often do you eat vegetables, fruit or berries?
0 p. Every day
1 p. Not every day

6. Have you ever taken medication for high blood pressure on regular basis?
0 p. No
2 p. Yes

7. Have you ever been found to have high blood glucose (eg in a health examination, during an illness, during pregnancy)?
0 p. No
5 p. Yes

8. Have any of the members of your immediate family or other relatives been diagnosed with diabetes (type 1 or type 2)?
0 p. No
3 p. Yes: grandparent, aunt, uncle or first cousin (but no own parent, brother, sister or child)
5 p. Yes: parent, brother, sister or own child

Total Risk Score
☐ The risk of developing type 2 diabetes within 10 years is

Lower than 7	Low: estimated 1 in 100 will develop disease
7–11	Slightly elevated: estimated 1 in 25 will develop disease
12–14	Moderate: estimated 1 in 6 will develop disease
15–20	High: estimated 1 in 3 will develop disease
Higher than 20	Very high: estimated 1 in 2 will develop disease

Please turn over

APPENDIX: QUALITY OF LIFE QUESTIONNAIRE (SF-36V2 HEALTH SURVEY)

Quality of Life Questionnaire (SF-36v2 Health Survey)

This survey asks for your views about your health, how you feel and how well you are able to do your usual activities. Answer every question by checking the appropriate response. There are no right or wrong answers. If you are unsure about how to answer a question, please give the best answer you can.

Date of visit *dvdate* ☐☐/☐☐/☐☐☐☐

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

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

2. Compared to one year ago, how would you rate your health in general now? *rthlth*

Much better	Somewhat better	About the same	Somewhat worse	Much worse
1 ☐	2 ☐	3 ☐	4 ☐	5 ☐

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

	Yes, limited a lot	Yes, limited a little	No, not limited at all
a. **Vigorous activities, such as running, lifting heavy objects, participating in strenuous activities.** *vgract*	1 ☐	2 ☐	3 ☐
b. **Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf** *mdract*	1 ☐	2 ☐	3 ☐
c. **Lifting or carrying groceries** *lcgroc*	1 ☐	2 ☐	3 ☐
d. **Climbing several flights of stairs** *cmstair*	1 ☐	2 ☐	3 ☐
e. **Climbing one flight of stairs** *csstair*	1 ☐	2 ☐	3 ☐
f. **Bending, kneeling, or stooping** *bdknstp*	1 ☐	2 ☐	3 ☐
g. **Walking more than a mile** *wlkml*	1 ☐	2 ☐	3 ☐
h. **Walking several hundred yards** *wlkyd*	1 ☐	2 ☐	3 ☐

	Yes, limited a lot	Yes, limited a little	No, not limited at all
i. Walking one hundred yards *wlkoyd*	1 ☐	2 ☐	3 ☐
j. Bathing or dressing yourself *bthdrs*	1 ☐	2 ☐	3 ☐

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 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. Cut down on the amount of time you spent on work or other activities *cuttm*	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
b. Accomplished less than you would have liked *dolss*	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
c. Were limited in the kind of work or other activities *lmtknd*	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
d. Had difficulty performing the work or other activities (for example, it took extra effort) *diffwrk*	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐

5. 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. Cut down the amount of time you spent on work or other activities *ecuttm*	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
b. Accomplished less than you would like *edolss*	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
c. Did your work or activities less carefully than usual *elsscr*	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups? *extent*

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

7. How much bodily pain have you had during the past 4 weeks? *pnxtnt*

None	Very mild	Mild	Moderate	Severe	Very severe
1 ☐	2 ☐	3 ☐	4☐	5 ☐	6 ☐

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

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

9. These questions are about how you feel and how things have been with you during the past 4 weeks. 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 Past 4 weeks....	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Did you feel full of life? *flife*	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
b. Have you been very nervous? *nervs*	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
c. Have you felt so down in the dumps that nothing could cheer you up? *edown*	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
d. Have you felt calm and peaceful? *ecalm*	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
e. Did you have a lot of energy? *fenrgy*	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
f. Have you felt downhearted and depressed? *edprss*	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
g. Did you feel worn out? *wrnout*	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
h. Have you been happy? *ehppy*	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
i. Did you feel tired? *etred*	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐

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

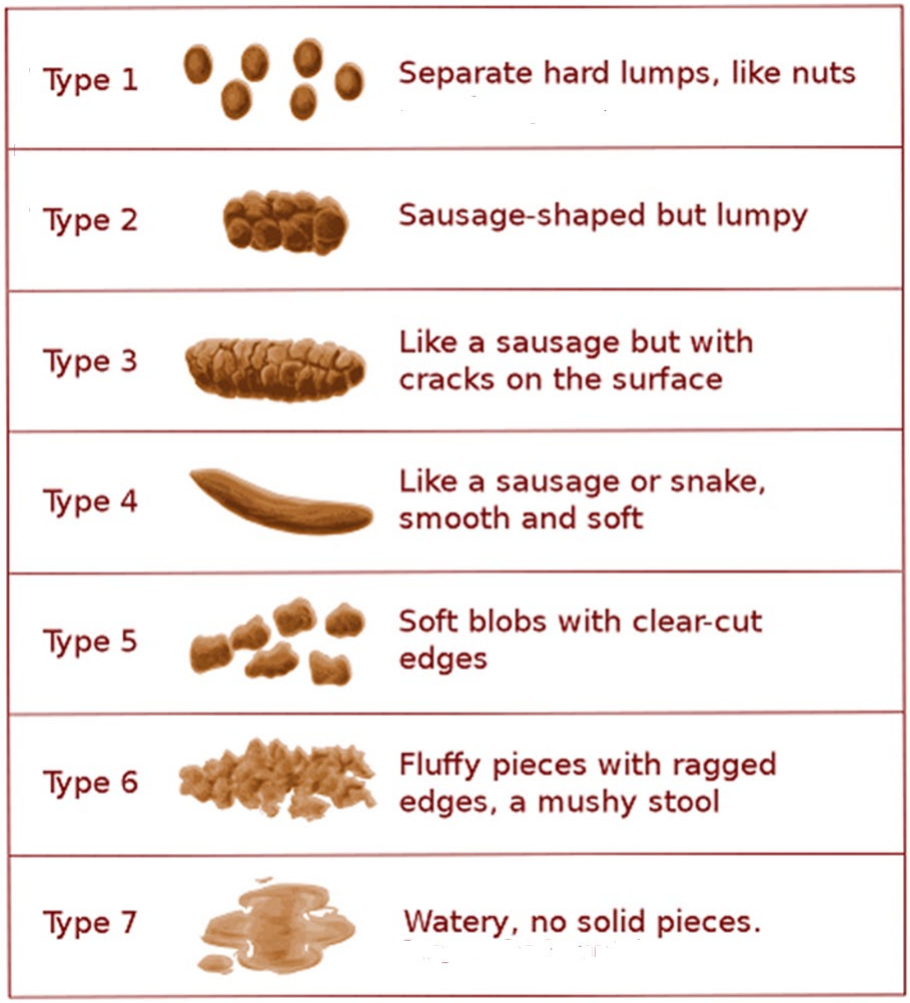
All of the time	Most of the time	Some of the time	A little of the time	None of the time
1 ☐	2 ☐	3 ☐	4 ☐	5 ☐

11. How TRUE or FALSE is each of the following statements for you?

	Definitely True	Mostly True	Don't Know	Mostly False	Definitely False
a. I seem to get sick a little easier than other people *esysck*	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
b. I am as healthy as anybody I know *hlthy*	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
c. I expect my health to get worse *hlthwrs*	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐
d. My health is excellent *hlthgd*	1 ☐	2 ☐	3 ☐	4 ☐	5 ☐

APPENDIX: BRISTOL STOOL FORM SCALE (Lewis & Heaton, 1997)

Bristol Stool Form Scale (English for United States)



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