**High Value Nutrition – Priority Research Program**

**Peak Nutrition for Metabolic Health [PANaMAH]**

**The New Zealand Synergy Study: Diabetes Prevention in Asian Chinese**

a residential study in pre-diabetic Asian Chinese and

European Caucasian cohorts

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| **Study protocol** |

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**Summary**

The pan-New Zealand National Science Challenge High Value Nutrition (NSC-HVN) programme aims to identify established and novel blood, urine and faecal microbiome markers of increased type 2 diabetes (T2D) risk in Asian Chinese individuals; and in turn to undertake nutritional interventions that target these biomarkers and so decrease T2D progression in high risk individuals.

Tranche 1 of the HVN Programme investigated the susceptibility to T2D in individuals who may be at increased risk of the disease even whilst outwardly-lean, likely due to deposition of fat in ‘risky’ sites such as visceral adipose depots, liver and pancreas. This phenomenon is termed the *Thin-on-the-Outside-Fat-on-the-Inside* (TOFI) profile.

A cohort of Asian Chinese and European Caucasian adults were enrolled into the TOFI-Asia study. They were of wide age range, bodyweight, body mass index (BMI) and diabetic risk based on blood glucose concentrations. Detailed phenotyping of the cohort confirmed adverse lipid deposition in metabolically ‘risky’ sites, and worse dysglycaemia and dyslipidaemia in Asian Chinese at an earlier age and lower BMI compared to matched European Caucasians (Sequeira et al., 2019) In addition global (untargeted LC-MS) metabolomic analyses identified novel blood biomarkers of diabetes susceptibility, with a unique Chinese ‘fingerprint’ of markers. Notably these markers differed significantly between the 2 ethnicities (Wu et al., 2019). The cause of this dichotomy in the metabolome is not known, but we hypothesise a number of causes including:

1. ethnicity, i.e., genetic background, and/or
2. pathology, i.e., dysglycaemia/diabetes risk, and/or
3. lifestyle, i.e., different habitual diets

A series of clinical studies will be now be conducted, firstly to investigate the cause(s) of Asian/Caucasian separation of plasma metabolome as outlined above; and secondly to validate these biomarkers as sensitive to nutrition (diet) intervention in a study design utilising NZ food products.

These studies in an Asian Chinese cohort will be conducted under full dietary control, using a typical Chinese diet. The first study proposed is a ‘cause and effect’ **residential study**, with all food items provided and compliance carefully monitored. This requires the specialised long stay facilities of the University of Auckland Human Nutrition Unit (HNU). In order to validate outcomes from this strictly controlled intervention in a closer to ‘real world’ setting a second study will be conducted **‘free living’ at home** where participants again have all food items provided by the HNU but where 100% compliance to diet cannot be ensured, and other variables must also be accounted for e.g. variable exercise regimes/energy expenditure.

Response of Asian Chinese to the interventions will be compared with a European Caucasian cohort. It is hypothesised that if diet is the primary driver of this unique metabolome ‘fingerprint’, biomarker dichotomy will be significantly diminished and in turn replaced with overlapping metabolite features between the 2 ethnicities.

**Background**

***Type 2 diabetes - a global health concern***

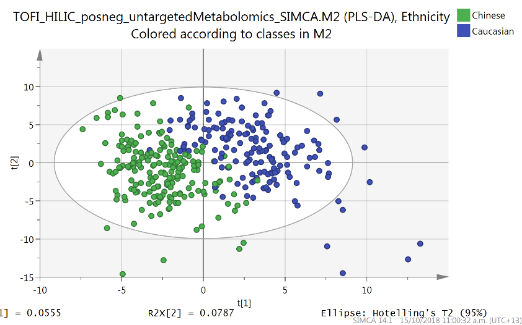
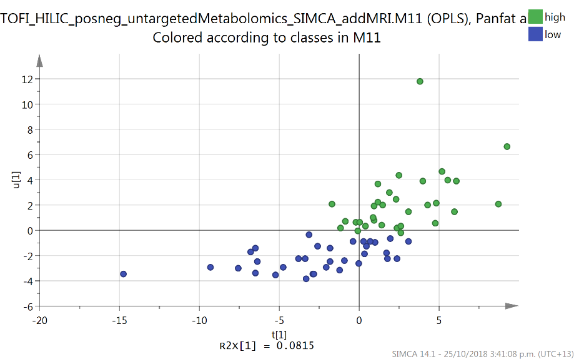
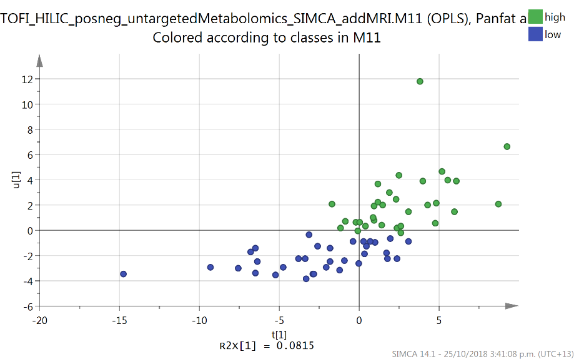
Diabetes and Adverse Metabolic Health have become a critical healthcare and economic problems globally (World Health Organization, 2016) with over 90% of those diagnosed with diabetes classified as type-2 diabetes (T2D) (American Diabetes Association, 2012). The prevalence of T2D has dramatically escalated from 110 million people reported in 1994, to 382 million in 2013, and is predicted to further increase to 592 million by 2035 (Zimmet, Magliano, Herman, & Shaw, 2014). This metabolic disorder is responsible for the death of approximately 1.5 million people annually and is a risk factor for cardiovascular disease (CVD), killing 13 million people worldwide each year and accounting for 25% of all-cause mortality (Lozano et al., 2012). T2D is not only a health concern for developed nations such as Western Europe, North America and Oceania, but recent alarming evidence shows increasing prevalence in developing Asian countries as well (Alberti & Zimmet, 2014); particularly China (Wang et al., 2017; Xu et al., 2013; Yan et al., 2012; Zuo, Shi, & Hussain, 2014), where T2D prevalence is estimated to reach 69% by 2030 in comparison to 20% in developed western countries (Shaw, Sicree, & Zimmet, 2010). New Zealand is no exception to these trends with the number of individuals with T2D having more than doubled in the last 10 years, with >250,000 individuals with this metabolic disease (Ministry of Health, 2016).

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 (Sniderman, Bhopal, Prabhakaran, Sarrafzadegan, & Tchernof, 2007; Tene et al., 2018) rather than ‘safe’ superficial subcutaneous adipose compartments. Increased visceral adiposity - particularly 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 (Dickinson, Colagiuri, Faramus, Petocz, & Brand-Miller, 2002; Liew, Seah, Yeo, Lee, & Wise, 2003) thereby worsening risk of T2D.

In Phase 1of the High Value Nutrition programme our research team phenotypeda unique cohort of ~400 Chinese & Caucasians 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 an age- and BMI-matched Caucasian cohort (Sequeira et al., 2019), 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 (Wu et al., 2019). We also identified the characteristic pattern of lipid ‘overspill’ into pancreas(Singh, Yoon, Poppitt, Plank, & Petrov, 2017) and liver in a sub-cohort of women who underwent magnetic resonance imaging for pancreas fat and magnetic resonance spectroscopy to identify liver fat.

Early findings from the TOFI-Asia study have shown the critical importance of cohort selection in studies investigating metabolic risk, with major ethnicity differences in the plasma metabolome between these 2 groups (**Figure 1a**) (Sequeira et al., 2019; Wu et al., 2019). The differences were characterised in Asian Chinese by low amino acid (AA) and related-metabolites including tyrosine, kynurenine, methyl-L-lysine and high 2-aminoisobutyrate, hydroxyl-L-proline; also notably low p-Cresol sulfate a gut microbial metabolite of aromatic AA origin.

In addition, when analysed independent of age, gender and BMI, fasting plasma glucose (FPG) was shown to be significantly correlated with 35 metabolites in Caucasians and as many as 120 in Asian Chinese, with the metabolite DG(38:5) being most strongly associated with FPG in Caucasians and DG(36:3) with FPG in Asian Chinese. Fifteen metabolites associated with FPG were common to both ethnicities including glycerolipid species and LC-MS-measured glucose.

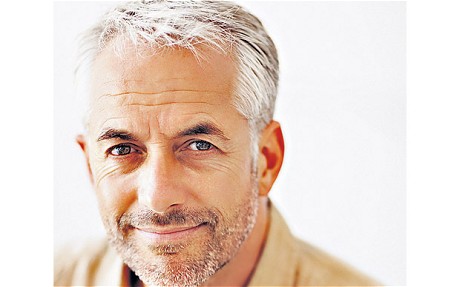


**Fig 1b**

OPLS

**Fig 1a**

PLS-DA

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In the TOFI-Asia cohort we have also shown a characteristic metabolite profile for high ectopic fat within the pancreas (**Figure 1b**), with low AAs including methionine, asparagine, taurine, histidine. High pancreas fat was also strongly correlated with high liver fat.

***Faecal Microbiome***

The faecal microbiome has also been identified as a source of biomarkers of interest in T2D, and may play a role in prediabetes and susceptibility to dysglycaemia. A common observation in cohort studies is a shift in the relative proportions of the 2 major bacterial phyla present in the faecal microbiome, the Firmicutes and Bacteroidetes (Murphy et al., 2010; Schwiertz et al., 2010) Notably however, there is little consensus as to the direction in which the ratio between these 2 phyla shifts. Individual bacterial ‘species’, or OTUs, may be more informative. Or it may be necessary to identify functional differences of the bacteria observed in order to unravel this complex issue (Ley, et al., 2005). Beyond compositional changes, the microbial effects on whole-body metabolic processes may be significant (Cani, Osto, Geurts, & Everard, 2012) including effects on insulin resistance and glucose metabolism.

***Epigenetics, SNPs and miRNAs***

Dietary intake have been shown to regulate the activity of genes (García-Segura, Pérez-Andrade, & Miranda-Ríos, 2013; Hardy & Tollefsbol, 2011), without modifying DNA (Feil & Fraga, 2012), by using specific signalling molecules called microRNA (miRNA). These miRNAs are small noncoding endogenous RNA molecules that modulate the expression of target genes, at the transcriptional or post transcriptional level, by binding to complementary regions in the coding messenger RNAs (mRNAs) resulting in mRNA decay of the target gene (Sluijter & Pasterkamp, 2017). Therefore they play an important role in a range of biological processes including adipocyte differentiation (Krützfeldt & Stoffel, 2006), glucose metabolism and lipid metabolism with altered circulating miRNA levels reported in obesity and diabetes (Heneghan, Miller, & Kerin, 2010; Nielsen et al., 2012; Pescador et al., 2013). Furthermore, single nucleotide polymorphisms (SNPs) act as biological markers to locate the genes associated with disease. Several SNPs have been related to obesity and diabetes (Saucedo et al., 2017). Epigenetic markers such as DNA methylation sites have also been associated with different levels of visceral and superficial adiposity (Lin et al., 2017) and independently associated with diabetes, smoking exposure, plasma HDL-cholesterol and lipoprotein (a) levels (Wahl et al., 2017). Hence a measure of circulating miRNAs, known DNA methylation sites as well as particular SNPs could be useful as a putative biomarker of disease susceptibility may be of interest to the program.

***Immunomodulation***

Changes in immune cells can alter immune function influencing progression to T2D (O'Neill et al., 2016). Human peripheral blood mononuclear cells (PBMCs) are key drivers of the immune response to dietary intervention and comprise several classes of immune cells, including T cells (~70%), B cells (~15%), monocytes (~5%), dendritic cells (~1%) and natural killer (NK) cells (~10%). Prior global transcriptomic analyses of PBMCs have successfully elucidated some underlying mechanisms of diabetes (Collares et al., 2013) and characterised the immune response in obese individuals to identify those ‘at-risk’ to developing adverse metabolic health (Richard et al., 2017). PBMCs, from plasma, can therefore provide a comprehensive overview of overall immune status and could aid in identifying (i) and validating potential biomarkers that may be modulated by dietary intervention, (ii) differentially activated mechanisms/pathways of T2D disease progression.

***Resting Energy Expenditure & Insulin-Induced Thermogenesis***

Dietary intake stimulates metabolism (metabolic rate), with modifications in dietary composition shown to alter substrate utilisation (Hall et al., 2007). Diet induced thermogenesis (DIT) or the postprandial metabolic rate refers to the increase in energy expenditure above basal fasting levels that occurs after ingesting food. DIT, along with basal metabolic rate (BMR) and activity induced thermogenesis, is one of three primary components of daily energy expenditure (DEE). DIT accounts for approximately 10% of DEE, and is considered to play an important role in the development and/or maintenance of obesity and adverse metabolic health. Significant reductions in DIT in age-matched individuals with obesity compared to lean counterparts are reported (de Jonge et al., 1997). Concomitantly, obese individuals are also shown to have 3–5% lower mean BMR which may contribute to weight gain in these individuals (Astrup et al., 1999). In contrast to BMR, studies have shown that postprandial thermogenesis (PPT) is actually reduced in the development of both impaired glucose tolerance (Weyer, Bogardus & Pratley, 1999) and overt diabetes (Golay et al., 1982). Therefore, it is plausible that measures of both fasting and postprandial energy expenditure may be utilised to characterise response of ‘at-risk’ individuals (Granata et al., 2002) to dietary interventions, as well as any improvements resulting thereof. Indeed, a secondary, longitudinal analysis of data collected from Pima Indians (a cohort with heightened prevalence of T2D) has identified a significant decline in insulin-induced thermogenesis as individuals progressed from normal glucose tolerance (11.7%) to impaired glucose tolerance (4.2%) to T2D (2.6%) over the course of 5.1 ± 1.4 years (Weyer, Bogardus & Pratley, 1999). This study also suggested that the majority of PPT attenuation occurred early in the stages of glucose intolerance, specifically in the transition from normal- to impaired glucose tolerance. However few robust prospective investigations have been conducted in this area and the mechanisms by which these changes occur remain unknown.

***Residential diet controlled studies at the Human Nutrition Unit***

The residential metabolic facility at the University of Auckland Human Nutrition Unit is unique within New Zealand and one of only a handful of such facilities worldwide. It conducts ‘gold standard’ dietary controlled randomised nutrition intervention trials, where all foods consumed are provided, with participants resident at the Unit for up to 4 weeks at a time depending on study protocol. Background habitual diet varies considerably between individuals, even those following similar lifestyle and dietary advice, unlike pharmaceutical trials where the background intake of prescription drugs is known and controlled. Rigorous control of the background diet in a residential study allows a cause and effect relationship to be established between a dietary intervention and selected outcome measures (e.g. blood biomarkers).

Participants are kept in energy balance through the intervention, to ensure body weight remains stable. There is no weight gain or loss. Diets are personalised for each individual with respect to energy (kJ/MJ) content; and all participants are provided with 3 meals per day comprising breakfast, lunch and dinner; and between meal snacks. Breakfast and dinner are consumed under supervision of the HNU research team to ensure 100% compliance. However, in order for participants to attend work or study if required, lunch meals and snacks are provided by the HNU but may be eaten away from the research unit. Participants are requested not to upload photographs or other information about the diets or the study Protocol to social media. Independent assessment of dietary compliance is carried out using the urine nitrogen balance method.

In these controlled trials, intermediary risk factors and biomarkers for T2D and associated metabolic health outcomes are measured throughout the intervention through analysis of blood, urine and faecal samples, collected using methods that include simple venepuncture and venous cannulation.

**Study Objective**

The objective of this study is to investigate the effect of dietary intervention on established and novel biomarkers of T2D in a high risk pre-diabetic population; and in addition to determine whether the dichotomy in plasma metabolome biomarkers observed between Asian Chinese and European Caucasian adults (recruited across a wide age range, bodyweight, body mass index (BMI) and diabetic risk) in the TOFI-Asia study is due to different habitual diets between the 2 ethnicities.

This will be investigated by conducting a 2 week, residential, full dietary control, 3 treatment intervention in 2 x 10 Asian Chinese and 1 x 10 European Caucasian cohorts. Participants will be overweight or obese, and with raised fasting blood glucose (FBG) indicative of prediabetes.

**2.1 Study Aims**

The aims of the study are to assess:

1. change in plasma and urine biomarkers, including established clinical and novel metabolome profiles, between baseline (0w) and 2 weeks (2w)

* in a prediabetic Asian Chinese cohort and an age- and BMI- matched European Caucasian cohort
* with complete dietary control during a 2 week residential study at the University of Auckland Human Nutrition Unit

1. change in established plasma and urine clinical markers of obesity and type 2 diabetes between baseline (0w) and 2 weeks (2w)
2. change in faecal microbiome between baseline (0w) and 2 weeks (2w)
3. **Methods**

**3.1 Trial Design**

This trial will be a 2 week, fully diet controlled, parallel design, 3 treatment intervention, conducted within the Human Nutrition Unit (HNU) residential facility.

**Fig. 1** shows the 3 treatment study design.

Two main comparisons will be conducted:

1. Effect of dietary intervention (Diet comparison) will be assessed in an Asian Chinese cohort by comparison of (i) ***healthy diet – control***, which adheres to NZ and Chinese national dietary guidelines for good metabolic health, vs. (ii) ***healthy diet – NZ synergy diet***, which adheres to NZ and Chinese national dietary guidelines for good metabolic health plus inclusion of a combination of New Zealand origin foods hypothesised to further improve glycaemic/metabolic health outcomes

2. Effect of ethnicity (Ethnicity comparison) will be assessed through comparison of an (i) ***Asian Chinese: healthy diet - control***, which adheres to NZ and Chinese national dietary guidelines for good metabolic health vs. (ii) ***Caucasian European:*** ***healthy diet - control***, which adheres to NZ and Chinese national dietary guidelines for good metabolic health.

**Figure 1. Intervention Treatment Arms**

**Diet** comparison

**Ethnicity** comparison

Asian Chinese, n=10: health diet - control

Asian Chinese, n=10: healthy diet - NZ synergy diet1

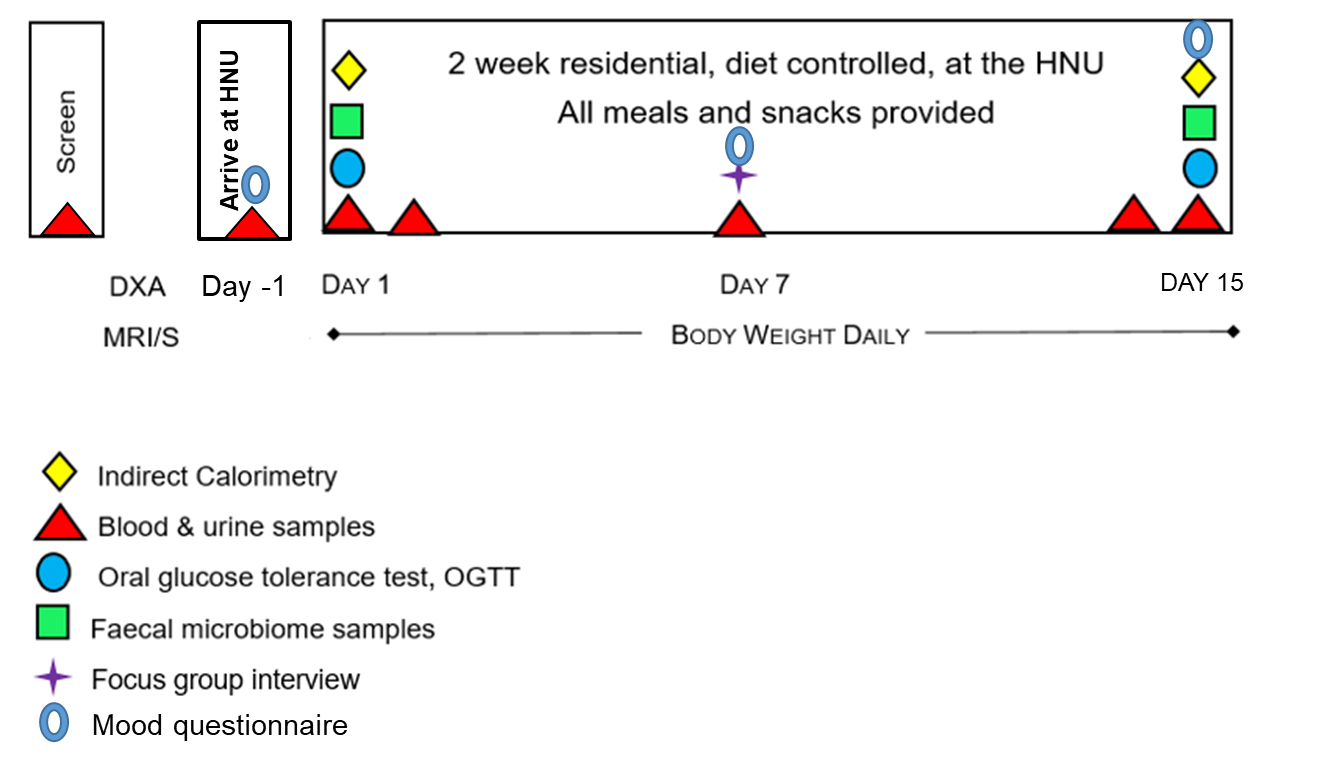
Caucasian European, n=10: healthy diet - control

2 week intervention, residential intervention, full diet control

1NZ synergy diet will comprise 5-7 food products with expected synergistic health benefits, combined to optimise positive effects on metabolic health.

**Fig. 2** shows the 2 week study protocol for all study participants, highlighting key assessments of body weight, resting (and post prandial) metabolic rate by indirect calorimetry, DXA and MRI/MRS body composition, blood, urine and faecal samples and single focus group interview.

**Figure 2. Study Protocol**

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Baseline only measures

DXA, dual energy x-ray absorptiometry (body composition)

MRI/S, magnetic resonance imaging/spectroscopy (pancreas fat, liver fat)

**Diet**

The intervention diet will be based on a typical Asian Chinese-style diet. The diet will adhere to New Zealand Ministry of Health healthy eating (Ministry of Health, 2015), and national guidelines of China (pagoda).

***Healthy diet – control:*** healthy eating guidelines. The macronutrient compositionwill be comprise ~55 en% carbohydrate (CHO), with <10en% added sugars; ~30en% total fat, with <10en% saturated fat; ~15en% protein, with <120g/week red meat.

***Healthy diet – NZ synergy***: healthy eating guidelines matched to control arm above, optimised for improved metabolic health through inclusion of a combination of New Zealand origin foods comprising 5-7 food products in synergy.

The diet will contain both animal and plant products, hence vegetarian and vegan participants are excluded. All foods, snacks and beverages will be provided by the Human Nutrition Unit. All foods, snacks and beverages must be consumed on a daily basis, with all foods for the day consumed before 12am midnight. No other foods or beverages will be allowed during the 14 days study.

Daily energy intake will be calculated based on 1.4 x predicted basal metabolic rate (BMR), using gender- and age-specific equations. This is equivalent to a sedentary day, where physical activity level (PAL) is 1.4 x BMR. Participants will be kept in energy balance throughout the intervention to ensure no weight gain or loss.

***5 day rotating diet***: The diet will comprise a 5 day rotating menu, which will repeat throughout the 14 days study:

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Monday | Tuesday | Weds | Thurs | Friday | Sat | Sun |
| Week 1 | Menu 1 | Menu 2 | Menu 3 | Menu 4 | Menu 5 | Menu 1 | Menu 2 |
| Week 2 | Menu 3 | Menu 4 | Menu 5 | Menu 1 | Menu 2 | Menu 3 | Menu 4 |

The diet will be prepared in 0.5MJ increments (e.g. 6, 6.5, 7, 7.5, 8, 8.5MJ) and hence individualised for each participant. An example is shown below:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | BMR (MJ) | PAL | TEE (MJ) | Diet allocation (MJ) |
| Male | 7.4 | 1.4 | 10.36 | 10.5 MJ |
| Female | 5.2 | 1.4 | 7.28 | 7.0 MJ |

PAL, physical activity level

TEE, calculated total daily energy expenditure

Assuming energy balance (EB): energy intake (EI) = energy expenditure (EE)

**Participants**

**Inclusion**

* Male and female
* Asian Chinese and European Caucasian
* Aged between 18-60 years
* BMI 24-40 kg/m2
* Fasting plasma glucose (FPG) in prediabetic range, 5.6 – 6.9 mmol/L
* Otherwise healthy, as per self-report
* Agreement to participate in a residential study

**Exclusion**

* Type 1 or type 2 diabetes mellitus
* Low serum iron and ferritin levels
* 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
* Recent body weight loss/gain >10% within previous 3 months or taking part in an active diet program; or current medications for weight loss
* Previous bariatric surgery
* Smoker, current or in previous 6 months
* Recreational drug user, 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 study, current or in previous 6 months
* Pregnant or breastfeeding women, current or in previous 6 months
* Considered unsuitable to participate by the PI
* Blood donation within previous 3 months.

**Participant Recruitment and Screening**

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

Data on gender, age, ethnicity, reported bodyweight and height, diabetes risk (FINDRISC, see Appendix) score, brief medical record, current medications, supplement intake and recent blood donation (<3 months) will be collected via telephone/online pre-screening questionnaire to ensure that inclusion/exclusion criteria are met prior to attending screening assessment at the HNU clinic.

Additionally, to ensure accuracy in recording medical information, participants will be required to bring with them to the HNU any prescriptions/list of medications from their General Practitioner (GP). In the event that participants are unsure of details of their medical history (health information) or medications we will check this with their GP (only following informed and written consent).

**Power calculation**

A *priori* modelling of sample size required to identify the change in plasma metabolomic biomarker ‘fingerprint’ in response to a 2 week dietary intervention as significant has been estimated using data from our previous TOFI\_Asia study. This was a cross sectional analysis where plasma metabolomics biomarkers were measured on 1 occasion (baseline) using state-of-the-art LC-MS in a cohort of ~400 Asian Chinese and European Caucasians. Using variance data from this study (plasma metabolites) and estimates of expected effect size (effect of diet over 2 weeks – dichotomous ‘fingerprint’ of metabolites between ethnicities converge or diverge or unchanged), response of the metabolites was modelled across a range of sample sizes. In order to be able to identify a significant difference between the 2 ethnicity ‘fingerprints’ using PLS-DA modelling, it was estimated that n=10 individuals was required in each of the 2 ethnicity cohorts.

All statistical models generated during data analysis in the residential study will be adjusted for potential confounding (if any) due to inclusion of both genders in the study design, which may influence the response/outcome variables of interest.

**Clinic Visits**

1. **Screening Visit**

All participants will be fasted overnight prior to attending the screening visit at the Human Nutrition Unit, 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, bodyweight, waist and hip circumference, BMI and blood pressure) will be recorded. A fasting blood sample will be conducted to assess fasting blood glucose and your blood iron and ferritin status.

Once enrolled, participants will be given a faecal collection kit, and requested to collect a sample for microbiome analyses, and to bring this sample to the clinic at the start of the 2 week residential phase. A dietary questionnaire (FFQ/24hr food record) will also be completed.

1. **Run-in phase/Baseline**

***Oral glucose tolerance test (OGTT)***

A 2 hr oral glucose tolerance test (OGTT) will be conducted to assess glycaemic response. Participants will visit the HNU in the early morning, prior to breakfast. A fasting venous blood sample will be collected for analysis of baseline plasma glucose. A 75g glucose drink will then be consumed immediately afterwards. A follow up venous blood sample will then be collected after 60 and 120 minutes for analysis of post challenge blood glucose concentrations.

***Body composition***

Total and compartmental body fat, pancreas fat and liver fat content will be characterised using dual-energy x-ray absorptiometry (DXA) scanning, and magnetic resonance imaging (MRI) and spectroscopy (MRS).

**Dualenergy 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 Body Composition Facility at the Department of Surgery, University of Auckland/Auckland City Hospital (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 minutes. 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. It will be performed using a respiratory-gated sequence (Bredella et al., 2010) and 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.

**Faecal Microbiome - overview**

A single faecal (sub) sample will be collected by participants on 2 occasions, immediately prior to start of study Day 1 and at the end of study Day 14, using standard HNU protocols. Participants will be given a home collection kit in order to bring a faecal (sub) sample to the HNU on Day 1. This includes two 5mL finger vials for sample storage, which is then sealed within a larger water filled pottle to ensure no external contamination, and frozen at -20°C. Samples will be transferred to -80oC storage until batch analysis at the end of the study. DNA extraction, amplification of bacterial 16S rRNA genes through PCR and library preparation will be undertaken, and DNA sequencing conducted using Illumina HiSeq or similar. Bioinformatics and statistical analyses of the obtained 16S rRNA gene sequences will be performed using a previously established pipeline. Quantitative PCR (qPCR) will determine potential proportional changes among key taxa and operational taxonomic units (OTU, ‘species’ level). Additionally regulation of the aryl hydrocarbon receptor (AhR), which is a ligand-activated transcription factor, that 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 V & Quintana FJ., 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, stool 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 and/or RNA will be extracted from stool 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 and/or ribosomal RNA gene sequencing). Sequencing will be undertaken by a commercial provider using next-generation sequencing technologies (e.g. Illumina HiSeq/NovaSeq). Bioinformatic and statistical analyses of the obtained sequences will be performed using previously established analysis pipelines. 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 studies, bacterial sequence data will be deposited (in a de-identified form) into a reference database at the time of publication in a peer-reviewed journal.

Although many gut microbes have thus far resisted cultivation on artificial media, cultivation of some members of the stool microbial community will be carried out. Isolation of these organisms will be attempted using standard laboratory growth media.

**Basal and post prandial energy expenditure - indirect calorimetry**

The cohort will be phenotyped in the fasted and postprandial state, in response to standardised 75g oral glucose drink (OGTT) at the start and end of study. 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 (VO2) and carbon dioxide (VCO2) production, e.g. *BMR at 30 minutes for and Post prandial at 2 hours*. Heart rate will be measured by a wireless chest belt, and blood pressure by a digital sphygmomanometer.

1. **Residential Phase: diet controlled - 2 weeks**

Participants will arrive at the HNU the day before the start of the study (Day -1) and will be resident under supervision at the Unit until the morning of Day 15 (end of study). Body weight is measured fasted on all days to monitor energy balance and ensure no weight gain/loss.

**Week 1**

Day -1 Arrival at HNU (+ faecal sample, diet questionnaire + blood sample collection + mood questionnaire)

Day 1 - Baseline

6.30am: bodyweight, spot urine sample; cannulation + baseline blood sample (t=-60)

6.45am: body temperature & blood pressure, start indirect calorimetry (30 min BMR, fasted)

7.30am: indirect calorimetry stopped (canopy removed), body temperature & blood pressure

7.40am: Collect baseline blood sample (t-5)

7.42-7.45am: Consume 75g OGTT glucose drink, t=0 min, 2-h OGTT indirect calorimetry (re-start)

8-9.30am: blood sample every 15 min, t=15min to t=90 min

9.45am: final blood sample t=120min, cannula removed, indirect calorimetry stopped (canopy removed), body temperature & blood pressure

10.00am: breakfast

1pm: lunch

4pm: mid-afternoon snacks,

7pm: dinner

10pm: bedtime

Day 2

6.45 am: bodyweight, blood sample, spot urine sample

7-8am: breakfast

10am: mid-morning snacks

1pm: lunch

4pm: mid-afternoon snacks

7-8pm: dinner

10pm: bedtime

Day 3 – full diet control

Day 4 - full diet control

Day 5 - full diet control

Day 6 - full diet control

Day 7

6.45 am: bodyweight, blood sample, 24-h urine sample

7-8am: breakfast

10am: mid-morning snacks, focus group interview, mood questionnaire

1pm: lunch

4pm: mid-afternoon snacks

7-8pm: dinner

10pm: bedtime

**Week 2**

Day 8 - full diet control

Day 9 - full diet control

Day 10 - full diet control

Day 11 - full diet control

Day 12 - full diet control

Day 13 - full diet control

Day 14

6.45 am: bodyweight, blood sample, spot urine sample, faecal sample (d14 or d15)

7am: breakfast

10pm: mid-morning snacks

1pm: lunch

4pm: mid-afternoon snacks,

7-8pm: dinner

10pm: bedtime

Day 15;

6.30am: bodyweight, spot urine sample; cannulation + baseline blood sample (t=-60)

6.45am: body temperature & blood pressure, start indirect calorimetry (30 min BMR, fasted)

7.30am: indirect calorimetry stopped (canopy removed), body temperature & blood pressure

7.40am: Collect baseline blood sample (t-5)

7.42-7.45am: Consume 75g OGTT glucose drink, t=0 min, 2-h OGTT indirect calorimetry (re-start)

8-9.30am: blood sample every 15 min, t=15min to t=90 min

9.45am: final blood sample t=120min, cannula removed, indirect calorimetry stopped (canopy removed), body temperature & blood pressure

10.00am: breakfast + mood questionnaire

END of STUDY

***An example of the Protocol of Day -1 (day before study begins) and Day 1 (start of study) is illustrated in the schematic below:***

|  |  |  |
| --- | --- | --- |
| **Day** | **Time** | **Measurements** |
| **-1** | **Arrival at HNU** | **faecal sample, diet questionnaire + blood sample collection + mood questionnaire** |
| **1** | **6:30 am** | bodyweight, spot urine sample; cannulation + baseline blood sample (time = - 60minutes) |
|  | **6:45 am** | body temperature & blood pressure, start indirect calorimetry (30 min BMR, fasted) |
|  | **7:30 am** | indirect calorimetry stopped (canopy removed), body temperature & blood pressure |
|  | **7:40 am** | Collect baseline blood sample (time-5) |
|  | **7:42 -7.45am** | Consume 75g OGTT glucose drink, time = 0 minutes, 2-h OGTT indirect calorimetry (re-start) |
|  | **8-9.30 am** | blood sample every 15 minutes, time = 15 minutes to time = 90 minutes |
|  | **9.45am** | final blood sample time = 120 minutes, cannula removed, indirect calorimetry stopped (canopy removed), body temperature & blood pressure |
|  | **10.00am** | Breakfast |
|  | **1.00 pm** | Lunch |
|  | **4.00 pm** | Mid-afternoon snacks |
|  | **7.00 pm** | Dinner |
|  | **10.00 pm** | Bedtime |

**RANDOMISATION TO THE STUDY**

All 10 European Caucasian participants will receive the healthy diet (control) while 20 Asian Chinese participants will be randomly allocated in a 1:1 ratio to receive either the healthy diet (control) or the healthy diet (NZ Synergy diet) over the two week intervention period.

The study will be a single blind study and the HNU has the maximum capacity/occupancy to conduct the study with 5 participants in residence. Following statistical consultation, with Dr Yannan Jiang at the Department of Statistics at the University of Auckland, the Clinical study will be conducted using a predetermined randomisation order as described below:

1. Firstly, the Asian Chinese will be randomised to either Healthy control or Healthy Synergy intervention arms
2. Secondly, the 10 participants from each of the 3 intervention arms will be randomly allocated to first (early phase) or second cohort (late phase) starting their treatment at different time points. This will ensure that any temporal/seasonal effects are minimised.

For example (the order may change but pre-determined),

Cohort 1: Caucasian control group (early start, n=5)

Cohort 2: Chinese control group (early start, n=5)

Cohort 3: Chinese intervention group (early start, n=5)

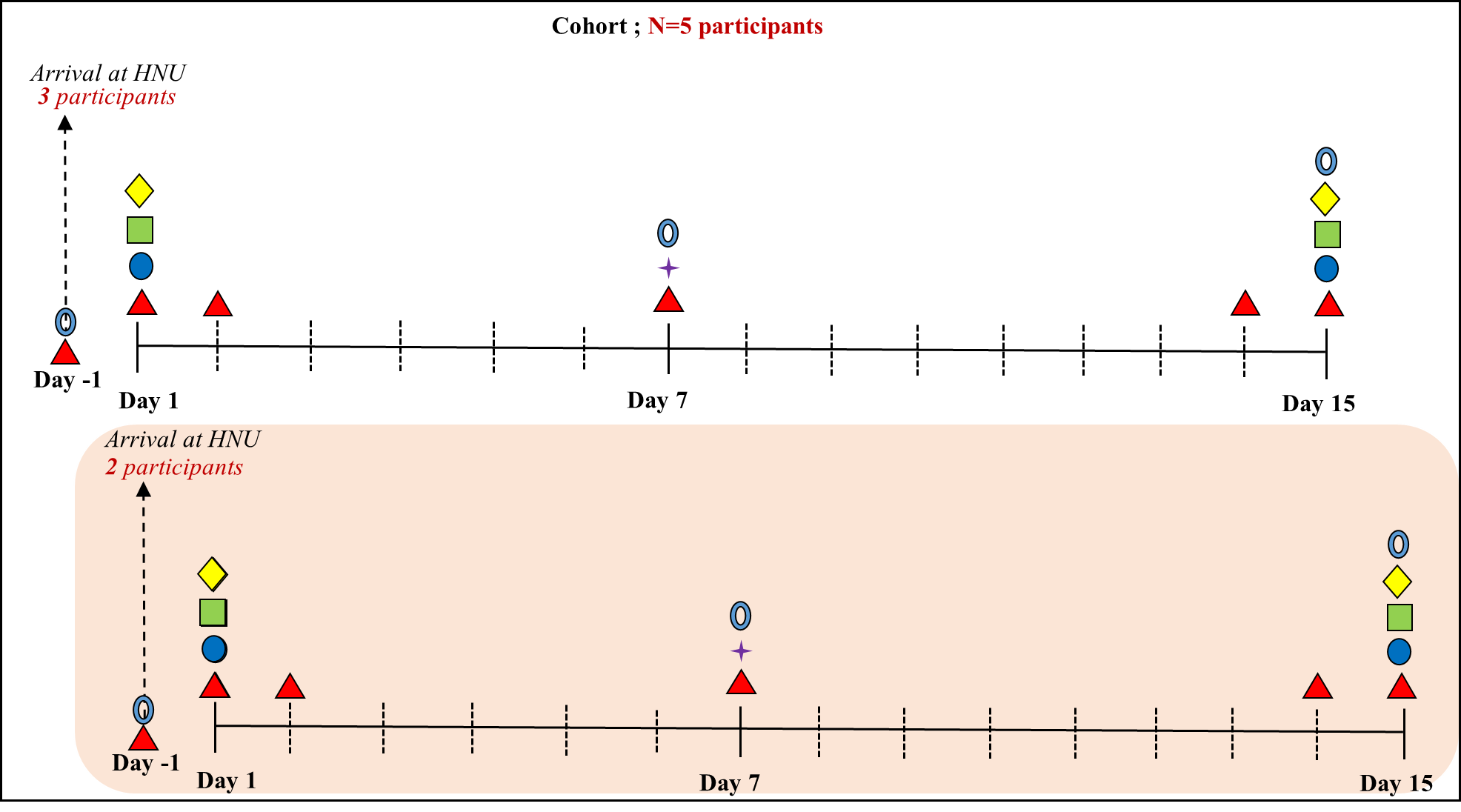
Cohort 4: Caucasian control group (late start, n=5)

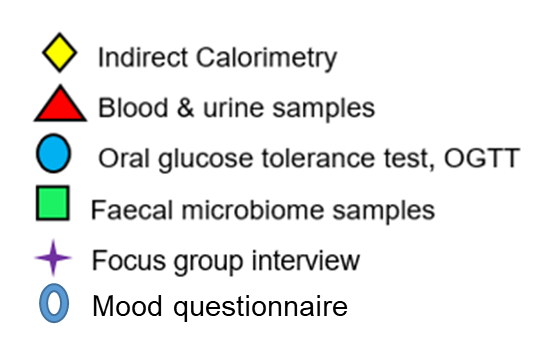
Cohort 5: Chinese control group (late start, n=5)

Cohort 6: Chinese intervention group (late start, n=5)

*\*For Caucasian participants, they will be randomly allocated to Cohort 1 (early start) or Cohort 4 (late start). \*\*For Chinese participants, they will first be randomised to either Healthy Synergy diet or Healthy control diet group (i.e. trial randomisation). They will then be randomly allocated to Cohort 2 or 5 if they are in the healthy control diet group, or Cohort 3 or 6 if they are in the Healthy Synergy diet group.*

The start of each cohort will be arranged such that 3 participants will begin the study a day before 2 participants, at to the HNU (as illustrated in Fig. 3); to total 5 participants in each cohort. The staggered start is adopted so that the fully controlled residential study can be conducted with 5 participants over the 2 week intervention period. Careful monitoring throughout the study by Research Staff will be followed, ensuring all Clinical trial protocols and standard operating procedures are followed from Day-1 to Day 15 of the study.

**Figure 3: Planned staggered start to the study for each cohort of 5 participants resident at the Human Nutrition Unit over the 2 week intervention period**

****

**Daily Activities**

Each participant is allocated an individual study/bedroom for the duration of the intervention. Participants are free to relax in the Unit throughout the day, following a sedentary protocol. They may undertake light activities (e.g. walking, cycling commuting) but are asked not to undertake strenuous physical activities (e.g. competitive or long-distance running).

**Compliance to Diet**

Compliance to diet will be monitored throughout the 14 day study. All foods, beverages and snacks will be provided. Breakfast and dinner meals will be consumed under supervision. Lunch and snacks may be eaten outside the HNU. No other foods will be allowed. Nitrogen balance will be measured on Day 7, based on 24-h urine collection.

**Thoughts/Insights about the Intervention and Residential Study**

During the study, a Survey and an Interview will be conducted by the Research Team to understand their needs, wants and motivation in relation to diet and health. This may help provide information regarding formulation and feasibility of diets designed for prevention of type 2 diabetes. The interview will be conducted with fellow participants resident at HNU as a Focus Group, and the survey will be administered to all participants in the form of an anonymous questionnaire consisting of 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 on the survey, or during the focus group. The focus group interview will last ~1hr and conducted on a single day (e.g. mid stay, Day 7), when at the same time the survey will be administered in an envelope to all participants resident at HNU 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 in the envelope, and can be handed in to the Researchers at any time. 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.

For all participants, baseline Visual Analogue Scale (VAS) ratings of their mood (Bond, A. and M. Lader.,1974) will also be measured using a simple questionnaire (questionnaire in Appendices). This questionnaire will take no more that 5-10 minutes to complete and will be part of their **Run In Phase** measurements. Participants will mark their responses by placing a vertical line across the 100-mm scale according to their subjective feelings (Fig 3). There is no correct or incorrect answer, it is just the participant’s personal opinion. They will rate how "alert" (represented by lines anchored by alert–drowsy, attentive–dreamy, lethargic–energetic, muzzy–clearheaded, well-coordinated–clumsy, mentally slow–quick witted, strong–feeble, interested–bored, incompetent–proficient); "calm" (calm–excited, tense–relaxed); and "content" (contented–discontented, troubled–tranquil, happy–sad, antagonistic–friendly, withdrawn–sociable), stressed, anxious and mentally fatigued they feel using standard VAS methods. The distance from the right hand side (R) or left hand side (L) of the line is measured in millimetres (or adjusted appropriately if the lines are not 100 mm) and the average score from the combined scales is used to compute a score for ‘alert’, ‘calm’, ‘content’, stressed, anxious and mentally fatigued respectively. These VAS scores will additionally be measured mid way during the study and at the end of the two week study period.

*Please indicate how you feel at this time by placing a mark on each line below:*

****

**Figure 3. Example of VAS.**

**Outcome Variables**

***1ry outcome***

* plasma metabolome profile

***2ry outcomes***

* clinical biomarkers including:
* fasting plasma glucose (FPG)
* postprandial 2-h glucose/OGTT
* HbA1c
* fasting insulin
* C-peptide
* urine metabolome profile

***Other outcomes***

* resting metabolic rate and glucose induced thermogenesis
* blood pressure and body temperature
* faecal microbiome and AhR activity
* urine Nitrogen (dietary compliance)
* clinical biomarkers including:
  + lipid profile (TC, LDL-C, HDL-C, TAG)
  + liver function tests
  + inflammatory markers and PBMCs for immune profiling
  + T2D related peptides, including adiponectin, amylin, glucagon, GLP-1
  + epigenetic, SNP, miRNA markers of T2D

**Blood Samples**

Clinical parameters (glycaemic/metabolic health)

Metabolome

**Urine Samples**

Metabolome

Nitrogen balance (compliance)

**Faecal Samples**

Microbiome + AhR activity

**Specialised analyses**

**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.

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.

**Clinical markers**

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

**MiRna analyses**

Small RNAs (miRNAs) analyses are conducted at the Grafton Clinical Genomics (GCG), an IANZ accredited laboratory at the University of Auckland. miRNAs are extracted from plasma using commercially available RNA kits and analysed using the nCounter Analysis System (NanoString Technologies) and the nCounter Human v2 miRNA Panel containing probes for 798 unique miRNAs and several housekeeping genes.

**Immune Profiling**

Following blood sample collection, plasma will be collected and stored at -80°C. At the end of the study, peripheral blood mononuclear cells (PBMCs) will be isolated using Lymphoprep™ (STEMCELL Technologies Inc.) system, transported and batch analysed at Malaghan Institute of Medical Research, Wellington. Planned methods under investigation 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 de-convoluted 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 dietary 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.

**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 dayand 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.

Dual Energy X-Ray Absorptiometry - DXA uses a low dose of ionizing radiation, similar to the natural radiation exposure of a 1 hour aeroplane flight. The exposure to participants represents a very low risk. 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.

**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 2 week intervention.

**9. Data Retention**

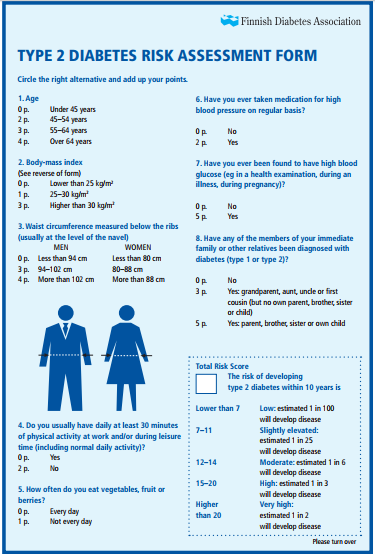
All data will be retained for a period of 10 years, or as stipulated by the NZ National Human Ethics Committee (HDEC).

**10. Clinical Trial Sites – The Human Nutrition Unit**

The study will be conducted at the University of Auckland Human Nutrition Unit ([www.humannutritionunit.auckland.ac.nz](http://www.humannutritionunit.auckland.ac.nz)) and the Department of Surgery Body Composition Unit at Auckland City Hospital.

**11. Appendices**

Appendix: findrisc form for the assessment of prediabetes



Appendix: questionnaire

**NEW ZEALAND DIETS FOR DIABETES PREVENTION**

**(SYNERGY STUDY): a residential nutrition intervention**

Date \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Day: 0 7 14 Study ID\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Please indicate how you feel **at this time** by placing a mark on each line below:

**DROWSY**

**ALERT**

**EXCITED**

**CALM**

**FEEBLE**

**STRONG**

**CLEAR-HEADED**

**MUZZY**

**CLUMSY**

**WELL COORDINATED**

**CLEAR-HEADED**

**MUZZY**

Date \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Day: 0 7 14 Study ID\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Please indicate how you feel **at this time** by placing a mark on each line below:

**ENERGETIC**

**LETHARGIC**

**DISCONTENTED**

**CONTENTED**

**TRANQUIL**

**TROUBLED**

**QUICK-WITTED**

**MENTALLY SLOW**

**RELAXED**

**TENSE**

**CLEAR-HEADED**

**MUZZY**

**DREAMY**

**ATTENTIVE**

Date \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Day: 0 7 14 Study ID\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Please indicate how you feel **at this time** by placing a mark on each line below:

**PROFICIENT**

**INCOMPETENT**

**SAD**

**HAPPY**

**FRIENDLY**

**UNFRIENDLY**

**BORED**

**INTERESTED**

**SOCIABLE**

**WITHDRAWN**

**CLEAR-HEADED**

**MUZZY**

Date \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Day: 0 7 14 Study ID\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Please indicate how you feel **at this time** by placing a mark on each line below:

***How STRESSED you feel at this moment?***

**Extremely**

**Not at all**

***How ANXIOUS you feel at this moment?***

**Extremely**

**Not at all**

***How MENTALLY FATIGUED you feel at this moment?***

**Extremely**

**Not at all**

**CLEAR-HEADED**

**MUZZY**

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