High Value Nutrition – Priority Program

P**ea**k **N**utrition for **Met**abolic **H**ealth [PANaMAH]

CHARACTERISING THE PRE-DIABETIC ASIAN AND CAUCASIAN PHENOTYPE:

THE 'TOFI' PROFILE

identifying biomarkers of diabetic susceptibility and resilience using a metabolomics platform

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Study protocol

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Public Summary

Weight gain and the poor metabolic health that develops as a consequence is rapidly becoming our most important global health condition. More than 1.5 *billion* adults worldwide struggle to control their weight and are now overweight or obese, with rates rapidly increasing throughout Asia including China where an estimated 30% of adults are struggling with their weight. Of these, 300 million have already been diagnosed with type 2 diabetes (T2D), the most common disease caused by overweight, and the numbers continue to rise.

The PEAK NUTRITION FOR METABOLIC HEALTH [PANAMAH] program is focused on Asian consumers who are looking for nutritional solutions to maintain good metabolic health throughout middle and older age. In particular the '*overweight and over forties'* looking to prevent T2D and to support heart health. Perhaps surprisingly, when matched with people of the same gender, age and body weight, Asian consumers are at much greater risk of poor metabolic health than Europeans, Maori or Pacific people. This highlights the need for the design, development and marketing of food and beverage products that are positioned at the high-value end of their product categories by New Zealand exporting businesses focused on Metabolic Health.

The reason why some individuals are more susceptible than others and what controls their T2D risk may lie in the storage of body fat. Gaining even small amounts of body weight can lead to the fat 'spilling over' from adipose tissue and into critical organs such as the muscle, liver and pancreas, which in turn may significantly increase risk of disease. Often known as TOFI – '<u>T</u>hin on the <u>O</u>utside, <u>F</u>at on the <u>I</u>nside' – ostensibly slim individuals can develop T2D whilst those who are morbidly obese may be resilient. A national collaborative research team across New Zealand will be conducting clinical studies supported by advanced molecular techniques to ask questions such as 'who is most at risk and why?', 'what are the early markers of disease, and do they differ in those resilient to T2D?', 'does lipid overspill matter?'; and how can these problems be targeted by food and beverages, particularly in key consumer groups for New Zealand food exports to Asia. T2D is a nutritional disease, caused primarily through poor lifestyle, and is able to be both prevented and treated through better nutrition. Understanding the mechanisms through which the disease is caused will help us to target the widespread problem of adverse Metabolic Health with nutritional solutions that can be employed by New Zealand food and beverage exporters.

The long-term aim of the Metabolic Health program within the High Value Nutrition program is to fast track NZ companies in their development of validated food and beverage health claims, that both satisfy national and International regulators in terms of the scientific validity of the food health relationship, and which also ultimately appeal to the tastes of the Asian market.

1. BACKGROUND

Metabolic health in Asia – obesity and type 2 diabetes

DIABETES AND ADVERSE METABOLIC HEALTH have become a critical healthcare and economic problem for Asia, with China in particular rapidly shouldering the largest global burden (Popkin 2008, WorldBank 2012, Chan, Zhang et al. 2014). Weight gain and an aging population have been identified as the most significant risk factors for adverse metabolic health, with the *'overweight and over forty'* at particular risk. Deaths from type 2 diabetes (T2D) worldwide are close to 1.5 million annually, in addition to which T2D is a major risk factor for later cardiovascular disease (CVD) including ischemic heart disease and stroke which together now kill more than 13 million people each year (Lozano, Naghavi et al. 2012, Murray, Vos et al. 2012).

The Asian consumer is rapidly becoming Westernised in food choices and lifestyle and equally rapidly has developed the myriad problems of weight gain, poor metabolic health, T2D and CVD so prevalent across other regions of the globe. Obesity has risen dramatically to 30% of today's Chinese adults (Yan, Li et al. 2012, Gordon-Larsen, Koehler et al. 2014), which means that over 1/5 of almost 2 billion overweight people globally now live in China (Ng, Fleming et al. 2014). The incidence of obesity-related metabolic diseases consequently has more than doubled over the past two decades (Yoon, Lee et al. 2006, Yang, Lu et al. 2010) and now represents the leading cause of morbidity, disability and mortality in Asian countries undergoing rapid economic and lifestyle changes including mainland China (He, Gu et al. 2005, Popkin 2008) where ~40% of children, 60% of adults, and >90% of older adults are affected by poor metabolic health, and a staggeringly high 300 million adults have been identified with T2D (Yan, Li et al. 2012). A further 500 million have pre-diabetes (Xu, Wang et al. 2013), with the International Diabetes Federation (IDF) predicting up to half a billion adults with T2D in China by 2030 (InternationalDiabetesFederation 2013), with already stretched healthcare costs predicted to increase exponentially.

With Asian children and adolescents also becoming overweight and obese, we will begin to see unprecedented numbers develop the metabolic health problems previously only seen in older adults.

Fig. 1 Prevalence of T2D in Asian populations
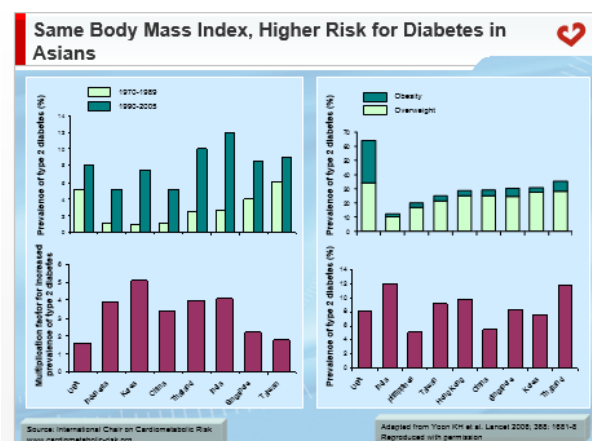
- Diabetes and metabolic syndrome are increasing rapidly in Asia.
- Asians, including Chinese, develop cardio-metabolic risk factors and type 2 diabetes at a considerably lower body mass index (BMI) and waist circumference (marker of abdominal obesity) than their Caucasian counter-parts.
- The mechanisms underpinning this remain poorly understood

Figure from Chan JC, Chinese University of Hong Kong, 2010 (Chan 2012)

Susceptible vs Resilient Phenotype

The underlying cause of adverse metabolic health remains only partly understood, with major gaps in our understanding of risk profile between individuals and/or populations where some are susceptible (Asian populations) and others more resilient (Caucasian populations) (Haldar, Chia et al. 2015). Ostensibly slim individuals with BMI within the lean range can develop T2D whilst those who are morbidly obese may be resilient. The molecular mechanisms responsible for key aspects of cardiometabolic disease, in particular T2D, are not known. Phenotypic risk factors, such as deposition of excess adipose tissue through weight gain and raised adverse biomarkers such as blood glucose, lipids or inflammatory markers, are established targets for prevention and treatment with F&B solutions; however the factors that regulate the fundamental pathophysiological events of diabetes - primarily insulin resistance (IR, inability of insulin to regulate blood glucose) and pancreatic β-cell degeneration (inability of the pancreas to secrete insulin) - have not as yet been determined.

There is also evidence that content and distribution of body fat are markedly different between ethnic groups, with a greater predisposition towards adiposity at higher BMI in Asians than in Caucasians (Haldar, Chia et al. 2015). In turn Asian populations are at greater risk of adverse metabolic health than their Caucasian counterparts, with little resilience again Western lifestyle and even modest weight gain appearing to lead to rapid metabolic dysregulation and development of T2D (Yoon, Lee et al. 2006). This may be a consequence of ectopic deposition of storage lipid in adverse 'high risk' anatomic sites (Nazare, Smith et al. 2012) including liver (Tota-Maharaj, Blaha et al. 2014), and development of the recently identified TOFI (Thin on the Outside, Fat on the Inside) profile (Thomas, Parkinson et al. 2012).

Thin on the Outside, Fat on the Inside - TOFI profile

Body mass index (BMI) and total adipose mass, whilst associated with dysglycaemia, are not adequate predictors of T2D and associated metabolic risk (Shah, Murthy et al. 2014), and underpinning susceptibility and resilience to adverse metabolic health may be site of lipid deposition (Mokdad AH 2001, Miyazaki Y 2002). The TOFI profile characterises individuals who have a phenotype of mild or moderate overweight, low to moderate peripheral adipose stores but high central adipose stores, with lipid both encapsulating and infiltrating into vital organs (Thomas, Parkinson et al. 2012). Whilst the majority of storage lipid is deposited within peripheral adipose tissue, there is some 'spill over' into ectopic sites such central adipose depots and critical organs such as liver, pancreas and muscle (Tota-Maharaj, Blaha et al. 2014, Zamboni, Rossi et al. 2014, Saponaro, Gaggini et al. 2015). This in turn been shown to significantly increase risk of metabolic disease. Deposition of lipid in a 'metabolically safe' place, ie peripheral adipose tissue, is advantageous and necessary since lipid consumed within the diet (~70g/day) is rarely completely oxidised and must be deposited into adipose stores to prevent hyperlipidaemia. Interestingly, individuals without peripheral adipose tissue (lipodystrophy) have an adverse metabolic profile similar to those individuals with too much adipose (obese) (Cortés and Fernández-Galilea 2015) with both phenotypes prone to lipid infiltration into critical organs amongst other similar characteristics such as insulin resistance (IR) despite the different aetiologies. There are a number of as yet unproven hypotheses with respect to ectopic lipid stores in the obese phenotype including the 'adipose tissue

expandability' hypothesis, where it is purported that large adipocytes have a limited capacity for expansion, forcing lipids to be stored in non-adipose ectopic depots (Johannsen, Tchoukalova et al. 2014).

Biomarker candidates

One of the key aims of PANaMAH is to identify metabolic profiles that characterise and predict susceptibility and resilience to T2D, in individuals both with and without the TOFI profile.

Metabolomics is a systems biology approach which can be employed to investigate low molecular weight metabolites within the metabolome of a chosen biological sample, eg. serum, urine (Dunn, Broadhurst et al. 2011). Its application in the investigation of impaired glucose tolerance (IGT) and T2D has led to the identification of new metabolic biomarkers (Griffin and Nicholls 2006, Oresic, Simell et al. 2008, Gall, Beebe et al. 2010, Wang-Sattler, Yu et al. 2012, Anderson, Dunn et al. 2014); and whilst as yet relatively little work has been done on biomarkers candidates of early risk for T2D, there is evidence to suggest that CHO dysregulation, ie. hyperglycemia, may not be the earliest metabolic change. In turn, and recently reviewed by Anderson and colleagues (Anderson, Dunn et al. 2014), it is becoming clear from recent large trials of dipeptidyl peptidase-4 (DPP-4) inhibitors that complications of T2D are not prevented simply by careful glycaemic control.

Several early targets have been proposed as indicators of T2D risk which include CHO metabolites monosaccharide hexose; amino acids including glycine, phenylalanine, acetylcarnitine (biosynthesized from lysine, methionine), 2-Aminoadipic acid (2-AAA); lipids including short and longchain fatty acids, diglycerides, as well as choline-containing and other phospholipids (Wang, Larson et al. 2011, Wang-Sattler, Yu et al. 2012, Floegel, Stephan et al. 2013, Wang, Ngo et al. 2013, Anderson, Dunn et al. 2014). A recent publication by a PANaMAH co-investigator has identified multiple early defects in lipid regulation which emerge prior to markers of hyperglycemia in women identified at increased risk through a previous history of gestational diabetes (Anderson, Dunn et al. 2014). Identification of further candidates, with focus on biomarkers which are both predictive of and responsive to nutrient intervention, in susceptible and resilient individuals and ethnicities is a primary aim of the HVN program. Response to diet has been little investigated to date and is an area of significant research interest.

Within the PANaMAH program we additionally are interested in identifying other novel blood biomarkers that may contribute to susceptibility and resilience to T2D, in individuals both with and without the TOFI profile. Dietary intake have been shown to regulate the activity of genes (Hardy and Tollefsbol 2011, Garcia-Segura, Perez-Andrade et al. 2013), without modifying DNA (Feil and 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 and Pasterkamp 2017). Therefore they play an important role in a range of biological processes including adipocyte differentiation (Krutzfeldt and Stoffel 2006), glucose metabolism (Latouche, Natoli et al. 2016), lipid metabolism and appetite regulation

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(Deiuliis 2016) with altered circulating miRNA levels reported in obesity and diabetes (Heneghan, Miller et al. 2010, Nielsen, Wang et al. 2012, Pescador, Perez-Barba 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, Valencia et al. 2017). Epigenetic markers such as DNA methylation sites have also been associated with different levels of visceral and superficial adiposity (Lin, Lim et al. 2017) and independently associated with diabetes, smoking exposure, plasma HDL-cholesterol and lipoprotein (a) levels (Wahl, Drong 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.

Magnetic Resonance Imaging - MRI

Also of interest to the program is investigation of body composition, including the TOFI profile where Asian populations have been shown to have deleterious abdominal fat distribution relative to other ethnicities (Nazare, Smith et al. 2012), and where lean individuals (BMI <25kg/m2, based on WHO cut off) may be at significant risk of IR and T2D at a younger age than their Caucasian counterparts. There is growing evidence that ectopic fat accumulation into organs such as liver and pancreas can best be predicted from visceral accumulation, using MRI (Rossi, Fantin et al. 2011), although visceral fat is only one of many ectopic fat depots used when the subcutaneous adipose tissue cannot accommodate excess fat because of its limited expandability (Smith 2015).

In addition, assessment of specific organs can be undertaken. To determine increased liver fat content, liver biopsy is currently considered the gold standard, however non-invasive techniques such as magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), computed tomography (CT) and ultrasound can be used. Ultrasound and CT provide only qualitative information whereas MRS- or MRI-based methods are able to quantitate small volumes of fat accurately (Springer, Machann et al. 2010).

Response to nutrition intervention

There is considerable evidence that key features of adverse metabolic health do respond to nutrition intervention. Current physiological goals for individuals with T2D include maintaining as near normal blood glucose concentrations, lipid profile that lowers risk of macrovascular disease, and blood pressure within a healthy range (Evert, Boucher et al. 2013). There is a large literature underpinning food-interactions with established biomarkers of T2D (Riccardi, Rivellese et al. 2008, Kataoka, Venn et al. 2013) and cardiovascular disease (Keogh, Cooper et al. 2003, Jackson, Poppitt et al. 2012, Mann, McLean et al. 2013) which are readily modifiable. Core components of advice to prevent onset of T2D in individuals with prediabetes are prevention of weight gain (or weight loss if obese), control of blood glucose and insulin concentrations. Foods and food products with low content of refined carbohydrates, low glycemic-index (GI), low saturated fat, and high levels of dietary fibre have long been central to achieving these metabolic goals (Franz, Bantle et al. 2002). Fibre-rich products may facilitate weight control and improve glycaemic control (Riccardi, Rivellese et al. 2008, Kataoka, Venn

et al. 2013), and modification of physical structure of CHO foods may readily alter glycemic response (Riccardi, Rivellese et al. 2008, Kataoka, Venn et al. 2013).   In addition, and of particular relevance to NZ food exports, may be higher protein foods which modify body composition and glycaemic/insulin control (McGregor and Poppitt 2013), with demonstrable beneficial effect on known biomarkers (incl. Hb_{A1c}, fasting/postprandial glucose, insulin, lipids, blood pressure), as well as novel biomarkers of risk (eg. blood peptides, 2-AAA and islet amyloid) (Erdmann K 2008, Ricci-Cabello, Herrera et al. 2012, Nasri and Nasri 2013). Of interest also are extracts from horticultural crops (eg. berryfruits, kiwifruit) rich in antioxidant polyphenols are known to exhibit beneficial cardiovascular and anti-inflammatory activity.

Phase II of this program will investigate response of established and novel biomarkers, including metabolomic markers identified in Phase I, to nutrition intervention.

2. STUDY OBJECTIVES

The objective of this study is to:

1. Characterise the Asian and Caucasian prediabetic phenotype in individuals, both with and without the TOFI profile, using a metabolomics approach

2. Identify potential biomarker targets predicted to respond to nutrition intervention

2.1 STUDY AIMS

In a group of overweight Chinese Asian adults and a group of overweight European Caucasian adults:

(i) identify risk of T2D based on glycaemic response using an oral glucose tolerance test (OGTT) and/or longer-term marker of glycaemia Hb_{A1c}: healthy (resilient profile) vs prediabetic (susceptible profile)

(ii) identify blood biomarkers which characterise the resilient vs susceptible profile, using a metabolomics approach

(iii) characterise body composition using whole body scanning techniques, incl. DeXA and MRI, with focus on site of adipose deposition and lipid infiltration into key organs, to investigate the TOFI profile (subset of cohort)

(iv) characterise the resilient vs susceptible profile for T2D based on metabolomic biomarkers and body composition

(v) characterise ethnic differences in risk profile for T2D based on metabolomic biomarkers and body composition, including those predicted to respond to nutrition intervention in Phase II future research within the PANaMAH program (not included in this Protocol)

3. METHODS

TRIAL DESIGN

This will be a cross-sectional study where assessment of diabetic risk, determined by response to OGTT and/or Hb_{A1c}, and body composition will be measured on a single occasion.

Fig 2. Study Protocol

ASIAN CHINESE [N=200]	CAUCASIAN EUROPEAN [N=200]
OVERWEIGHT/OBESE	OVERWEIGHT/OBESE
(I) HEALTHY – RESILIENT	(I) HEALTHY – RESILIENT
(II) PREDIABETIC - SUSCEPTIBLE	(II) PREDIABETIC - SUSCEPTIBLE

Range of metabolic health risk, based on glucose control

- Healthy
- Prediabetic

3.5 PARTICIPANTS, N=400

Two hundred Asian Chinese and two hundred European Caucasian adults, stratified for BMI, will be recruited into this study from the wider Auckland region, based on the inclusion/exclusion criteria shown below:

Inclusion criteria

- Asian (Ethnic Chinese, incl. mainland China, Singapore, Malaysian, Hong Kong, Taiwan, plus Korea), and
- Caucasian (Ethnic European)
- adults; men and women
- aged 18-70 years
- overweight or obese; BMI 20-50kg/m^2
- T2D risk and glycaemic status assessed as either 'No current risk/healthy' or 'Prediabetic'

Exclusion criteria

- Recent body weight loss/gain >10%, within previous 3 months
- Recent bariatric surgery, within previous 6 months
- Significant current disease
- Pregnant or breastfeeding women
- Standard exclusions for DXA and MRI scanning techniques, including cardiac pacemaker

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

3.6 PARTICIPANT RECRUITMENT AND SCREENING

Recruitment for Asian Chinese and European adults will be conducted in the Auckland region. Interested individuals will be invited to contact the Human Nutrition Unit for written information on the study.

Data on gender, age, ethnicity, reported body weight and height, brief medical record, current medications will be collected via telephone screening questionnaire to ensure that inclusion/exclusion criteria are met prior to attendance at the research clinic.

3.7 CLINIC VISITS

Following explanation of the study and the opportunity to ask questions of the research team, each study participant will sign an informed consent form (ICF). The following assessments with then be conducted:

All participants, n=400

- Body weight (kg), lightly clad, measured in duplicate
- Height (m), measured in duplicate
- Waist circumference (cm), measured in duplicate
- Fasting venous blood sample
- Hb_{A1c} and/or OGTT, 2hr test
- Urine, spot sample

Subgroup, n=200

- Body composition assessment by DXA

Subgroup, n=100

- Adipose tissue and ectopic lipid deposition assessment by MRI
- Cardiorespiratory fitness test.

Subgroup, n=40

- Intravenous glucose tolerance test
- Microbiome test

Subgroup, n=50

- Single day nutrient challenges, to assess postprandial response of metabolomic biomarkers; cross-over study

All clinic visits will be conducted by research staff trained for each technique according to study SOPs; recorded within the site training log.

3.8 METHODOLOGIES

3.8.1 – FASTING ASSESSMENTS

3.8.1A METABOLOMICS PLATFORM

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 utilise a non-targeted mass spectrometry based (MS) approach to measure multiple metabolites from venous blood samples across a large dynamic range.

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 LipidSearch software package.

3.8.1B BODY COMPOSITION ASSESSMENT: FAT MASS, ADIPOSE TISSUE AND ECTOPIC LIPID STORAGE

Dual energy x-ray absorptiometry (DXA) and magnetic resonance imaging (MRI) will be performed on 1 occasion to assess body composition; with focus on adipose tissue site, ectopic lipid deposition and infiltration into key organs of liver, pancreas, muscle.

DUAL ENERGY X-RAY ABSORPTIOMETRY [DXA]: DXA is based on the three component model of body composition, and uses two x-ray energies to measure body fat mass, lean mass, and bone mineral. A rapid scan iDXA (GE-Lunar, Madison, WI) designed to allow scanning of larger individuals with high body weight and BMI will be used. 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 as well as regional fat deposition will be determined from DXA whole-body and segmental scans.

MAGNETIC RESONANCE IMAGING [MRI]: MRI utilises a combination of a strong magnetic field and radio frequency (RF) pulse to obtain detailed images without the use of ionising radiation. Due to the high accuracy of images and safety of the imaging technique, MRI is a commonly utilised method for the many detailed body composition studies.

CAMRI, UoA: The MAGNETOM Skyra™ is the latest generation of MRI technology with a high table weight limit of 250kg and large bore, hence is well suited to obese patients. The XQ gradients combine 45 mT/m peak amplitude with a slew rate of 200 mT/m/msec. Up to 204 simultaneously connected coil elements, in combination with the 48 independent parallel RF channels, allow for the flexible parallel imaging and support difficult applications.

An alternate method of computed tomography (CT) utilises computer-processed combinations of multiple x-ray images taken from different angles to produce cross-sectional tomographic images (=virtual 'slices') of the region of interest. Less preferable that MRI for participant studies due to the ionising radiation dose.

3.8.1C CARDIORESPIRATORY FITNESS TEST (CRF)

This is a short and simple bicycle test that assesses how quickly the heart rate increases when performing exercise (Heyward & Gibson, 2014). It is not a difficult test, with the amount of exercise similar to climbing a flight of stairs. Before taking the test, a questionnaire will need to be completed by participant to confirm that there are no cardiovascular risks preventing them from exercising, and blood pressure measured. Participants will be asked to cycle slowly on a stationary bike in the clinic for about 10-12 minutes. Participants will be asked to cycle at a specific speed and resistance for *3 minutes, and then to repeat this several times* (maximum 3 repeats) so that heart rate slowly increases. The exercise begins at a very low level and will increased in stages depending on personal fitness level. During the whole test heart rate will be recorded using a monitor that is attached to the index finger. A researcher will be with the participant throughout the test and will stop the test at any time because of signs of fatigue or changes in heart rate or any other symptoms experienced. Participants can also stop at any time if have any feelings of fatigue or any other discomfort. This test is approved by the American College of Sports Medicine for older adults, and most people find the test quite easy to do (ACSM's guidelines for exercise testing and prescription, 2010).

3.8.1D Intravenous glucose tolerance test (ivGTT)

Participant will be given a small dose of glucose (a sugar that is found in many foods/beverages) directly into a vein in the arm, and we will measure how well the

pancreas (which secretes the important hormone insulin) functions. This is called an intravenous glucose tolerance test (ivGTT). Bloods samples will be collected at regular intervals over the next 70 minutes. The ivGTT is a routine test which takes approximately 1 ½ hours in total to complete, and will be conducted by the HNU Research Nurse and an investigator from the HVN team. Participants will receive the glucose infusion slowly via a cannula. After the glucose infusion blood samples will be collected at intervals from another cannula inserted in the participant's other arm. Towards the end of the test at the 60 minutes mark, 5g of arginine bolus will be given to participants intravenously to see how much more insulin the pancreas can produce. This test is used very frequently in diabetes testing and is simple and quite easy to perform.

3.8.1E Microbiome test

The gut contains many millions of bacteria (gut bugs) which recent research have shown to possibly be associated with good or poor health. These bacteria are known as the 'microbiome'. There may be bugs which are associated with being either lean or overweight, and healthy or diabetic. This is a very new area of research and the TOFI study will help to determine whether microbiome has any effect on metabolic health. The test can be done on a very small stool sample. Participants will be given a faecal sample collection kit that they can take home and will be asked to bring it along with them during their visit to the HNU. All samples will be stored in a -80°C freezer.

3.8.1 – POSTPRANDIAL ASSESSMENTS: RESPONSE TO NUTRIENT CHALLENGE

Acute response of metabolomic biomarkers to nutrient challenge will be assessed using single day protocols, in a sub-set of individuals who have completed the fasting assessments outlined previously. Interventions will include test meals of high-CHO (e.g. glucose, OGTT), high-lipid (e.g. high fat muffin, mayonnaise), high-protein (e.g. dairy whey protein), and mixed meal challenges. Following a 10 hour fast, participants will consume a standard fixed nutrient meal and postprandial blood response will be assessed over a 2 to 6 hour period, with timing based on the nutrient of interest. Intervention will be conducted using a cross-over design where response to multiple meal challenges are assessed in a single individual.

Figure 3. Nutrient challenge protocol, example
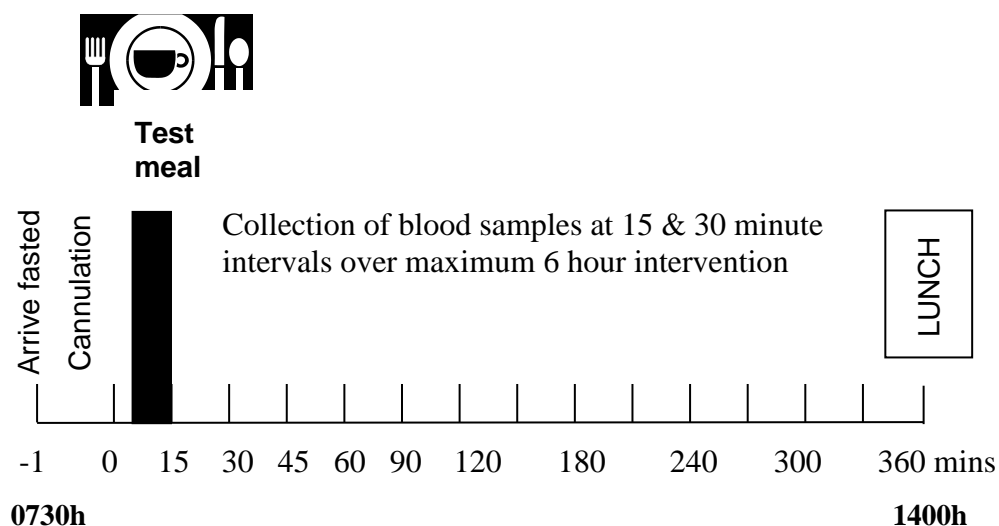
3.8.2 OUTCOME MEASURES

3.8.2A - FASTING ASSESSMENTS

Primary

Metabolomic profile, blood

Secondary

Body composition: total fat and lean mass, adipose tissue (AT) lipid deposition, ectopic non-AT lipid infiltration into key organs (e.g. liver, pancreas, muscle)

Other

Biochemistry panel including fasting glucose, insulin, Hb_{A1c}, full lipid profile, inflammatory cytokines (e.g. TNF-a, IL-6, IL-10), hsCRP, adiponectin, and associated obesity-related biomarkers

3.8.2B - POSTPRANDIAL ASSESSMENTS, INCL. NUTRIENT CHALLENGE

Primary

Metabolomic profile at fasted baseline and throughout postprandial assessment

Secondary

Body composition: total fat and lean mass, adipose tissue (AT) lipid deposition, ectopic non-AT lipid infiltration into key organs (e.g. liver, pancreas, muscle)

Other

Biochemistry panel including fasting glucose, insulin, Hb_{A1c}, full lipid profile, inflammatory cytokines (e.g. TNF-a, IL-6, IL-10), hsCRP, adiponectin, and associated obesity-related biomarkers

3.9 STATISTICAL ANALYSIS

Statistical power

It is not possible to determine *a priori* the sample size required to identify novel biomarkers of type 2 diabetes, hypothesised to differ between ethnicity and/or and body composition phenotype, using untargeted and/or targeted metabolomics methods since data on variance (metabolites) and expected differential effect size (ethnicity) are not available. However prior publications in this area indicate that a study population of n=400 will be adequate for this proof of principal investigation. Similar single ethnicity studies have been undertaken in populations of n=106 (Anderson, Dunn et al. 2014), n=129 (Oresic, Simell et al. 2008), and n=399 (Gall, Beebe et al. 2010).

3.10 RISKS AND BENEFITS

COLLECTION OF BLOOD SAMPLES, PHLEBOTOMY - a single venipuncture is required for this study, which may result in mild discomfort for the participant. Samples will be collected under supervision of a research nurse, and no adverse events are expected.

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.

3.11 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 Human Nutrition Unit and AgResearch.

3.12 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 at the clinic visit.

3.13 DATA RETENTION

All data will be retained for a period of 5 years, or as stipulated by the NZ national Human Ethics Committee (HDEC).

4. CLINICAL TRIAL SITES – THE HUMAN NUTRITION UNIT, AUCKLAND CITY HOSPITAL, AND AGRESEARCH

The study will be conducted at the University of Auckland Human Nutrition Unit (www.humannutritionunit.auckland.ac.nz) and the Department of Surgery Body Composition

Unit at Auckland City Hospital; Metabolomic analyses will be conducted at AgResearch, Palmerston North.

5. PROPOSED TIMELINES

Protocol finalised: mid February 2016

Human Ethics submission: end February 2016

Start recruitment: April/May 2016

Complete recruitment and study visits: December 2016

Start metabolomics analyses: January 2017

Reporting to HVN re Milestone: September 2017

6. REFERENCES

Anderson, S. G., W. B. Dunn, M. Banerjee, M. Brown, D. I. Broadhurst, R. Goodacre, G. J. S. Cooper, D. B. Kell and J. K. Cruickshank (2014). "Evidence that multiple defects in lipid regulation occur before hyperglycemia during the prodrome of type-2 diabetes." PLoS One 9: e103217.

Chan, J. C., Y. Zhang and G. Ning (2014). "Diabetes in China: a societal solution for a personal challenge." Lancet Diabetes Endocrinol adv e-publ Sept.

Chan, J. C. N. (2012). "Metabolic syndome: an Asian perspective." Chinese University of Hong Kong, presentation.

Cortés, V. A. and M. Fernández-Galilea (2015). "Lipodystrophies: adipose tissue disorders with severe metabolic implications." J Physiol Biochem 71: 471-478.

Deiuliis, J. A. (2016). "MicroRNAs as regulators of metabolic disease: pathophysiologic significance and emerging role as biomarkers and therapeutics." Int J Obes (Lond) 40(1): 88-101.

Dunn, W. B., D. I. Broadhurst, H. J. Atherton, R. Goodacre and J. L. Griffin (2011). "Systems level studies of mammalian metabolomes: the roles of mass spectrometry and nuclear magnetic resonance spectroscopy." Chem Soc Rev 40: 387–426.

Erdmann K, C. B., Schroder H. (2008). "The possible roles of food-derived bioactive peptides in reducing the risk of cardiovascular disease. Journal Nutr Biochem 2008;19(10):643-54.".

Evert, A. B., J. L. Boucher, M. Cypress, S. A. Dunbar, M. J. Franz, E. J. Mayer-Davis, J. J. Neumiller, R. Nwankwo, C. L. Verdi, P. Urbanski and W. S. Yancy (2013). "Nutrition therapy recommendations for the management of adults with diabetes." Diabetes Care 36: 3821-3842.

Feil, R. and M. F. Fraga (2012). "Epigenetics and the environment: emerging patterns and implications." Nat Rev Genet 13(2): 97-109.

Floegel, A., N. Stephan, Z. Yu, K. Mühlenbruch, D. Drogan, H. G. Joost, A. Fritsche and H. U. Häring (2013). " Identification of serum metabolites associated with risk of type 2 diabetes using a targeted metabolomic approach. ." Diabetes 62: 639-648.

Franz, M. J., J. P. Bantle, C. A. Beebe, J. D. Brunzell, J. L. Chiasson, A. Garg, L. A. Holzmeister, B. Hoogwerf, E. Mayer-Davis, A. D. Mooradian, J. Q. Purnell and M. Wheeler (2002). "Evidence-based nutrition principles and recommendations for the treatment and prevention of diabetes and related complications." Diabetes Care 25: 148-198.

Gall, W. E., K. Beebe, K. A. Lawton, K. P. Adam and M. W. e. a. Mitchell (2010). "Alphahydroxybutyrate is an early biomarker of insulin resistance and glucose intolerance in a nondiabetic population. ." PLoS One 5: e10883.

Garcia-Segura, L., M. Perez-Andrade and J. Miranda-Rios (2013). "The emerging role of MicroRNAs in the regulation of gene expression by nutrients." J Nutrigenet Nutrigenomics 6(1): 16-31.

Gordon-Larsen, P., E. Koehler, A. G. Howard, L. Paynter and etal (2014). "Eighteen year weight trajectories and metabolic markers of diabetes in modernising China." Diabetologia 57: 1820-1829.

Griffin, J. L. and A. W. Nicholls (2006). "Metabolomics as a functional genomic tool for understanding lipid dysfunction in diabetes, obesity and related disorders." Pharmacogenomics 7: 1095–1107.

Haldar, S., S. C. Chia and C. J. Henry (2015). "Body composition in Asians and Caucasians: comparative analyses and influences on cardiometabolic outcomes." Adv Food Nutr Res 75: 97-154.

Hardy, T. M. and T. O. Tollefsbol (2011). "Epigenetic diet: impact on the epigenome and cancer." Epigenomics **3**(4): 503-518.

He, J., D. Gu, X. Wu, K. Reynolds, X. Duan, C. Yao, J. Wang, C. S. Chen and J. Chen (2005). "Major causes of death among men and women in China." N Engl J Med **353**: 1124–1134.

Heneghan, H. M., N. Miller and M. J. Kerin (2010). "Role of microRNAs in obesity and the metabolic syndrome." Obes Rev **11**(5): 354-361.

InternationalDiabetesFederation (2013). "webpage - idf.org/sites/default/files/201212%20-%20IDF%20Submission%20Post%202015%20Environment%20Consultation.pdf."

Jackson, J. G., S. D. Poppitt and A. M. Minihane (2012). "The postprandial lipemic response and its associated phenotype: interrelationships between meal fat composition, physiological and genetic determinants." Atherosclerosis **220**: 22-33.

Johannsen, D. L., Y. Tchoukalova, C. S. Tam, J. D. Covington, W. Xie, J. M. Schwarz, S. Bajpeyi and E. Ravussin (2014). "Effect of 8 weeks of overfeeding on ectopic fat deposition and insulin sensitivity: testing the "adipose tissue expandability" hypothesis." Diabetes Care **37**: 2789-2797.

Kataoka, M., B. J. Venn, S. M. Williams, L. A. TeMorenga, I. M. Heemels and J. I. Mann (2013). "Glycaemic responses to glucose and rice in people of Chinese and European ethnicity." Diabetic Med **30**: e101-107.

Keogh, G. F., G. J. S. Cooper, T. B. Mulvey, B. H. McArdle, J. Munro, G. Coles and S. D. Poppitt (2003). "A randomized, controlled, cross-over study of the effect of highly beta-glucan-enriched barley on cardiovascular disease risk factors in mildly hypercholesterolaemic men." Am J Clin Nutr **78**: 711-718.

Krutzfeldt, J. and M. Stoffel (2006). "MicroRNAs: a new class of regulatory genes affecting metabolism." Cell Metab **4**(1): 9-12.

Latouche, C., A. Natoli, M. Reddy-Luthmoodoo, S. E. Heywood, J. A. Armitage and B. A. Kingwell (2016). "MicroRNA-194 Modulates Glucose Metabolism and Its Skeletal Muscle Expression Is Reduced in Diabetes." PLoS One **11**(5): e0155108.

Lin, X., I. Y. Lim, Y. Wu, A. L. Teh, L. Chen, I. M. Aris, S. E. Soh, M. T. Tint, J. L. MacIsaac, A. M. Morin, F. Yap, K. H. Tan, S. M. Saw, M. S. Kobor, M. J. Meaney, K. M. Godfrey, Y. S. Chong, J. D. Holbrook, Y. S. Lee, P. D. Gluckman and N. Karnani (2017). "Developmental pathways to adiposity begin before birth and are influenced by genotype, prenatal environment and epigenome." BMC Med **15**(1): 50.

Lozano, R., M. Naghavi, F. K. and etal (2012). "Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010." Lancet **380**: 2095-2128.

Mann, J., R. McLean and L. TeMorenga (2013). "Evidence favours an association between saturated fat intake and coronary heart disease." BMJ.

McGregor, R. A. and S. D. Poppitt (2013). "Milk proteins for improved metabolic health: a review of the evidence." Nutr Metab **3**: 46.

Miyazaki Y, G. L., Triplitt C et al. . (2002). "Abdominal fat distribution and peripheral and hepatic insulin resistance in type 2 diabetes mellitus." Am J Physiol Endocrinol Metab **283**: E1135–E1143.

Mokdad AH, F. E., Bowman BA et al. (2001). "Prevalence of obesity, diabetes, and obesity-related health risk factors." JAMA **289**: 76–79.

Murray, C. J., T. Vos, R. Lozano and etal (2012). "Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010." Lancet **380**: 2197-2223.

Nasri, R. and M. Nasri (2013). " Marine-derived bioactive peptides as new anticoagulant agents: a review." Curr Prot Peptide Sci **14**: 199-204.

Nazare, J. A., J. D. Smith, A. L. Borel, S. M. Haffner, B. Balkau, R. Ross, C. Massien, N. Alméras and J. P. Després (2012). "Ethnic influences on the relations between abdominal

subcutaneous and visceral adiposity, liver fat, and cardiometabolic risk profile: the International Study of Prediction of Intra-Abdominal Adiposity and Its Relationship With Cardiometabolic Risk/Intra-Abdominal Adiposity." Am J Clin Nutr **96**: 714-726.

Ng, M., T. Fleming, M. Robinson, B. Thomson, N. Graetz, C. Margono, E. C. Mullany, S. Biryukov and C. Abbafati (2014). "Global, regional and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013." Lancet **384**: 766-781.

Nielsen, L. B., C. Wang, K. Sorensen, C. H. Bang-Berthelsen, L. Hansen, M. L. Andersen, P. Hougaard, A. Juul, C. Y. Zhang, F. Pociot and H. B. Mortensen (2012). "Circulating levels of microRNA from children with newly diagnosed type 1 diabetes and healthy controls: evidence that miR-25 associates to residual beta-cell function and glycaemic control during disease progression." Exp Diabetes Res **2012**: 896362.

Oresic, M., S. Simell, M. Sysi-Aho, K. Nanto-Salonen, T. Seppanen-Laakso and e. al. (2008). "Dysregulation of lipid and amino acid metabolism precedes islet autoimmunity in children who later progress to type 1 diabetes." J Exp Med **205**: 2975–2984.

Pescador, N., M. Perez-Barba, J. M. Ibarra, A. Corbaton, M. T. Martinez-Larrad and M. Serrano-Rios (2013). "Serum circulating microRNA profiling for identification of potential type 2 diabetes and obesity biomarkers." PLoS One **8**(10): e77251.

Popkin, B. M. (2008). "Will China's nutrition transition overwhelm its health care system and slow economic growth?" Health Aff **27**: 1064–1076.

Riccardi, G., A. A. Rivellese and R. Giacco (2008). "Role of glycemic index and glycemic load in the healthy state, in prediabetes, and in diabetes." Am J Clin Nutr **87**: 269S-274S.

Ricci-Cabello, I., M. O. Herrera and R. Artacho (2012). "Possible role of milk-derived bioactive peptides in the treatment and prevention of metabolic syndrome." Nutr Rev **70**: 241-255.

Rossi, A. P., F. Fantin, G. A. Zamboni, G. Mazzali, C. A. Rinaldi, M. Del Giglio, V. Di Francesco, M. Barillari, R. Pozzi-Mucelli and M. Zamboni (2011). "Predictors of ectopic fat accumulation in liver and pancreas in obese men and women." Obesity **19**: 1747-1754.

Saponaro, C., M. Gaggini, F. Carli and A. Gastaldelli (2015). "The subtle balance between lipolysis and lipogenesis: a critical point in metabolic homeostasis." Nutrients **13**: 9453-9474.

Saucedo, R., J. Valencia, C. Gutierrez, L. Basurto, M. Hernandez, E. Puello, G. Rico, G. Vega and A. Zarate (2017). "Gene variants in the FTO gene are associated with adiponectin and TNF-alpha levels in gestational diabetes mellitus." Diabetol Metab Syndr **9**: 32.

Shah, R. V., V. L. Murthy, S. A. Abbasi, R. Blankstein, R. Y. Kwong, A. B. Goldfine, M. Jerosch-Herold, J. A. Lima, J. Ding and M. A. Allison (2014). "Visceral adiposity and the risk of metabolic syndrome across body mass index: the MESA Study." JACC Cardiovasc Imaging **7**: 1221-1235.

Sluijter, J. P. G. and G. Pasterkamp (2017). "MicroRNAs." The Swing Voters in Vascular Disease Waiting for a Program **120**(1): 5-7.

Smith, U. (2015). "Abdominal obesity: a marker of ectopic fat accumulation." J Clin Invest **125**: 1790-1792.

Springer, F., J. Machann, C. D. Claussen, F. Schick and N. F. Schwenzer (2010). "Liver fat content determined by magnetic resonance imaging and spectroscopy." World J Gastroenterology **16**: 1560-1566.

Thomas, E. L., J. R. Parkinson, G. S. Frost, A. P. Goldstone, C. J. Doré, J. P. McCarthy, A. L. Collins, J. A. Fitzpatrick, G. Durighel, S. D. Taylor-Robinson and J. D. Bell (2012). "The missing risk: MRI and MRS phenotyping of abdominal adiposity and ectopic fat." Obesity **20**: 76-87.

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Tota-Maharaj, R., M. J. Blaha, I. Zeb, R. Katz, R. Blankstein, R. S. Blumenthal, M. J. Budoff and K. Nasir (2014). "Ethnic and sex differences in fatty liver on cardiac computed tomography: the multi-ethnic study of atherosclerosis." Mayo Clin Proc **89**: 493-503.

Wahl, S., A. Drong, B. Lehne, M. Loh, W. R. Scott, S. Kunze, P. C. Tsai, J. S. Ried, W. Zhang, Y. Yang, S. Tan, G. Fiorito, L. Franke, S. Guarrera, S. Kasela, J. Kriebel, R. C. Richmond, M. Adamo, U. Afzal, M. Ala-Korpela, B. Albetti, O. Ammerpohl, J. F. Apperley, M. Beekman, P. A. Bertazzi, S. L. Black, C. Blancher, M. J. Bonder, M. Brosch, M. Carstensen-Kirberg, A. J. de Craen, S. de Lusignan, A. Dehghan, M. Elkalaawy, K. Fischer, O. H. Franco, T. R. Gaunt, J. Hampe, M. Hashemi, A. Isaacs, A. Jenkinson, S. Jha, N. Kato, V. Krogh, M. Laffan, C. Meisinger, T. Meitinger, Z. Y. Mok, V. Motta, H. K. Ng, Z. Nikolakopoulou, G. Nteliopoulos, S. Panico, N. Pervjakova, H. Prokisch, W. Rathmann, M. Roden, F. Rota, M. A. Rozario, J. K. Sandling, C. Schafmayer, K. Schramm, R. Siebert, P. E. Slagboom, P. Soininen, L. Stolk, K. Strauch, E. S. Tai, L. Tarantini, B. Thorand, E. F. Tigchelaar, R. Tumino, A. G. Uitterlinden, C. van Duijn, J. B. van Meurs, P. Vineis, A. R. Wickremasinghe, C. Wijmenga, T. P. Yang, W. Yuan, A. Zhernakova, R. L. Batterham, G. D. Smith, P. Deloukas, B. T. Heijmans, C. Herder, A. Hofman, C. M. Lindgren, L. Milani, P. van der Harst, A. Peters, T. Illig, C. L. Relton, M. Waldenberger, M. R. Jarvelin, V. Bollati, R. Soong, T. D. Spector, J. Scott, M. I. McCarthy, P. Elliott, J. T. Bell, G. Matullo, C. Gieger, J. S. Kooner, H. Grallert and J. C. Chambers (2017). "Epigenome-wide association study of body mass index, and the adverse outcomes of adiposity." Nature **541**(7635): 81-86.

Wang-Sattler, R., Z. Yu, C. Herder, A. C. Messias, A. Floegel, Y. He, K. Heim, M. Campillos, C. Holzapfel, B. Thorand and E. al (2012). "Novel biomarkers for pre-diabetes identified by metabolomics." Mol Sys Biol **615**: doi:10.1038/msb.2012.1043.

Wang, T. J., M. G. Larson, R. S. Vasan, S. Cheng, E. P. Rhee, E. McCabe, G. D. Lewis, C. S. Fox, P. F. Jacques, C. Fernandez, C. J. O'Donnell, S. A. Carr, V. K. Mootha, J. C. Florez, A. Souza, O. Melander, C. B. Clish and R. E. Gerszten (2011). "Metabolite profiles and the risk of developing diabetes." Nature Med **17**: 448-453.

Wang, T. J., D. Ngo, N. Psychogios, A. Dejam, M. G. Larson and etal (2013). "2-Aminoadipic acid is a biomarker for diabetes risk." J Clin Invest **123**: 4309-4317.

WorldBank (2012). "Toward a healthy and harmonious life in China: stemming the rising tide of non-communicable diseases." webpage - worldbank.org/content/dam/Worldbank/document/NCD_report_en.pdf.

Xu, Y., L. Wang, J. He, Y. Bi, M. Li, T. Wang, L. Wang, Y. Jiang, M. Dai and J. Lu (2013). "Prevalence and control of diabetes in Chinese adults." JAMA **310**: 948-958.

Yan, S., J. Li, S. Li, B. Zhang, S. Du, P. Gordon-Larsen, L. Adair and B. Popkin (2012). "The expanding burden of cardiometabolic risk in China: the China Health and Nutrition Survey." Obesity Reviews **13**: 810–821.

Yang, W., J. Lu, J. Weng, W. Jia, L. Ji, J. Xiao, Z. Shan, J. Liu and H. Tian (2010). "Prevalence of diabetes among men and women in China." N Engl J Med **362**: 1090–1101.

Yoon, K.-H., J.-H. Lee, J.-W. Kim, J. H. Cho, Y. H. Choi, S. H. Ko, P. Zimmet and H. Y. Son (2006). "Epidemic obesity and type 2 diabetes in Asia." Lancet **368**: 1681–1688.

Zamboni, M., A. P. Rossi, F. Fantin, S. L. Budui, E. Zoico, G. A. Zamboni and G. Mazzali (2014). "Predictors of ectopic fat in humans." Curr Obes Rep **3**: 404-413.