The Health for Life in Singapore (HELIOS) Study

PROTOCOL VERSION 5.0 (01 10 2021)

HELIOS
Health for Life in Singapore

LEE KONG CHIAN SCHOOL OF MEDICINE
NANYANG TECHNOLOGICAL UNIVERSITY SINGAPORE
Imperial College London

National Healthcare Group
Adding years of healthy life

Imperial College London

PROTOCOL TITLE

The Health for Life in Singapore (HELIOS) Study: Proposed design and development

PRINCIPAL INVESTIGATORS

No	Name	Organisation
1	Professor John **Chambers**	NTU Lee Kong Chian School of Medicine (Singapore)
2	Associate Professor Joanne **Ngeow** Yuen Yie	NTU Lee Kong Chian School of Medicine (Singapore)
3	Professor Paul **Elliott**	Imperial College London (UK)
4	Dr. **Lee** Eng Sing	National Healthcare Group (Singapore)
5	Dr. Jimmy **Lee**	National Healthcare Group (Singapore)
6	Professor Elio **Riboli**	Imperial College London (UK)

PROJECT LOCATION

1. Primary location: The HELIOS Screening Centre

Health Screening Centre, Level 18, Clinical Sciences Building, NTU Lee Kong Chian School of Medicine
11 Mandalay Road, Singapore 308232

2. Secondary location (data analysis)

School of Public Health, Imperial College London, Medical Building, Norfolk Place, London, United Kingdom W2 1PG.

PROJECT DESCRIPTION

The Health for Life in Singapore (HELIOS) Study is a state-of-the-art prospective cohort which will investigate the aetiology of complex diseases in Singapore. Approximately 3,500 Singaporean participants from the three main ethnic groups (Chinese, Malay, and Indian) will be recruited in the initial phase, with the aim of extending the study to include up to 10,000 participants, subject to funding and IRB review. At the baseline visit, comprehensive phenotype information will be collected from each participant, comprising health and lifestyle questionnaires, and extensive clinical and physical measurements, including: anthropometry, blood pressure, lung function (spirometry), electrocardiogram (ECG), arterial stiffness, 3-D carotid ultrasound, ophthalmology assessment, physical fitness test, physical activity monitoring (accelerometer), hand grip strength, cognitive function, and dual energy X-ray absorptiometry (DXA) scan. In addition, biological samples (blood, urine, saliva, stool, and skin tape) will be collected. Participants will then be followed up long-term and occurrences of health related outcomes will be recorded. The breadth of phenotypic measurements collected, together with biological samples will enable the investigation of the complex interrelationships between environmental, lifestyle and genetic factors on subsequent disease risk. The HELIOS study will be a uniquely rich resource for medical research across a wide range of disciplines for both the current and next generations of biomedical researchers.

1 Contents

2 AIMS

General aim of the study:
To investigate the epidemiology, natural history and determinants (environmental, lifestyle and genetic) of the diseases that are important to the Singapore population, including metabolic diseases such as diabetes and obesity, cardiovascular disease and its complications, neurological diseases, autoimmune disease, infectious and chronic respiratory diseases.

Our specific aims include:

- Initiate a prospective cohort of 3,500 participants, with the aim of extending the study to include up to 10,000 participants subject to funding and IRB review.
- Complete extensive baseline phenotyping of study participants, including clinical characteristics, functional testing, imaging, and molecular profiling.
- Store biological samples of blood, urine, saliva and stool for future molecular analyses
- Carry out long-term follow-up of participants for new onset disease
- Use the data generated to investigate the major determinants of ill-health and well-being in Singapore.
- Develop sub-studies, such as early disease patient cohorts for specific diseases which are public health priorities in the Singaporean population.
- Thereby create a uniquely rich resource for medical research across a wide range of disciplines for both the current and next generation of biomedical researchers.

3 RESEARCH PLAN

3.1 Background and Rationale

Singapore has one of the fastest ageing populations in Asia - between 2004 and 2014 the proportion of the population aged 65 years and over rose from 8.4% to 12.4%[1], and is projected to increase to 25% in 2030[2]. The current life expectancy in Singapore is 80 years for men and 84 years for women[3]. In recent years, increasingly unhealthy lifestyles have been adopted by the Singaporean population, evidenced by the rising prevalence of overweight and obesity. Currently, an estimated 29.3% of adult Singaporeans (34.5% of men and 24.3% of women) are classified as overweight, with 1 in 9 (10.8%) classified as obese[4].

As a consequence, the prevalence of ageing and lifestyle related chronic diseases have increased. In 2013, cardiovascular diseases and cancer were responsible for 61% of all deaths in Singapore[5]. A considerable burden has also been placed on the country's healthcare system by increasing numbers of patients living with the consequences and complications of incurable long-term health conditions or chronic diseases. In 2010, an estimated 216,922 years of life were lost as a result of disability, with diabetes (18%), vision disorders (8.1%), Alzheimer's and dementia (7.1%), and hearing disorders (5.4%) being the greatest contributors to this total[4].

The healthcare costs caused by these demographic and lifestyle changes are considerable and make disease prevention a priority. It is therefore an urgent priority to identify aetiological risk factors for these complex diseases so that appropriate preventative and treatment strategies can be implemented. Large population prospective cohorts with collection of extensive phenotype measurements and biological material are essential to understand the complex interrelationships between environmental and lifestyle factors with genetic factors on risk of subsequent disease. A key strength of prospective cohort studies is that measurements of environmental and lifestyle exposures are collected at baseline before the onset of the disease(s) of interest, therefore avoiding possible selection and other biases, including problems of reverse causality. Biological samples collected at baseline and stored can be analysed at a later stage to compare biochemical and metabolic characteristics of participants who later on do and do not develop disease. These baseline data enable the investigation of a wide range of potential risk factors in relation to disease occurrence, whether they have prognostic value for different diseases, and whether their association with disease might be causal. Further, by collecting data on prevalent cases of disease and ill-health at baseline, it is possible to investigate predictive biomarkers of disease progression and outcome to inform treatment strategies and personalised medicine.

Building on our experience from other large scale population cohorts, such as the European Prospective Investigation into Cancer and Nutrition (EPIC), UK Biobank, LOLIPOP study (migrant South-Asians), Airwave study (UK police forces) and the Qatar Biobank, the Lee Kong Chian School of Medicine and the National Healthcare Group (NHG) in partnership will establish the HELIOS study, with the initial aim of recruiting ca. 3,500 men and women aged 30-84 years from the Singapore population at the time of visit. During the baseline recruitment visit, participants will complete health and lifestyle questionnaires, and will undergo a broad range of clinical and physical measurements, including a state-of-the-art imaging module comprised of a 3-D carotid ultrasound, DXA scans for bone density and body composition, and comprehensive optical imaging. Additionally, participants will complete a physical fitness test and have activity levels over a 7 day period monitored through the use of accelerometer devices. The collection of

such an extensive range of phenotypic measurements allied with the collection of biological samples will provide the foundation for a powerful and comprehensive resource for investigations into the aetiology and pathogenesis of diverse disease outcomes, with potential to improve health and advance healthcare in Singapore and beyond.

3.2 **Innovation**

The breadth of phenotypic measurements proposed is at the leading edge internationally. Three other recent population cohorts - UK Biobank, German National Cohort, and the Qatar Biobank – have collected a similar range of measurements. The proposed imaging modules (3-D carotid ultrasound, total body DXA, and optical) are a state-of-the-art component in population cohorts that will enable the identification of pre-clinical disease phenotypes that will aid prognostic and preventative research. The biological material collected will allow high-throughput techniques in the areas of genomics, epigenomics, transcriptomics, proteomics, and metabolomics to be utilised. Similarly, the collection of saliva and stool samples will allow the relationships between oral and gut microbiota and disease to be investigated in Asia on a large-scale. With its multi-ethnic population, and highly advanced healthcare and research infrastructure, Singapore is an ideal location to carry out such studies. Future findings will inform and guide healthcare and public health practices not only in Singapore but elsewhere in the region and worldwide.

The population cohort will enable investigations of participants with early disease phenotypes. This approach will allow the study of the natural history of disease and prognostic biomarkers.

3.3 **Study Participant**

3.3.1 ***Population of Singapore***

The proposed cohort will recruit men and women aged 30-84 years old, aiming to recruit a cohort with age and sex distribution that approximates to the Singapore general population. In order to facilitate the study of differences across ethnic groups, the HELIOS study will oversample Indian and Malay participants such that the distribution of participants is approximately 60% Chinese, 20% Indian, and 20% Malay.

3.3.2 ***Inclusion criteria***

- Singaporean citizens and permanent residents aged 30-84 years at the time of visit
- Be able to read and write in at least one of the following languages: English, Chinese, Malay or Tamil (The participant is allowed to bring along someone over the age of 18 to help themcomplete the study questionnaire).

3.3.3 ***Exclusion criteria***

- Pregnancy (self-reported or by pregnancy test). We will carry out a pregnancy test on all women aged <55 years old (at no cost) before entering the study; a positive pregnancy test will exclude participation in this study.
- Breastfeeding
- Acute illnesses
- Recent major surgery (within the last 3 months)
- Unable or unwilling to give informed consent

3.3.4 ***Proposed sample size***

The power to detect associations between environmental and lifestyle exposures and health outcomes increases as a function of sample size[6], and as such it is optimal to collect data on the largest number of participants possible. However, within the constraints of cost and feasibility, there is typically a trade-off between study size and depth of phenotyping. The proposed sample size of the initial cohort is ca. 3,500 participants, with a view to recruiting up to 10,000 participants. The proposed sample size is based on a number of factors including feasibility, cost, prevalence of risk factors, incidence of the major common non-communicable diseases, and range and depth of the extensive phenotypic data and measurements proposed.

The expected number of prevalent disease cases in 10,000 participants of the HELIOS study based on the age-specific prevalence of the Singapore National Health Survey from 30-84 years is ~1,100 cases of type-2 diabetes, ~1,100 cases of obesity, ~2,400 cases of hypertension, and ~1,700 cases of hypercholesterolemia [7]. Among the healthy HELIOS participants at recruitment, it is expected that ~800 will develop Type-2 Diabetes in the next 10 years, ~800 will develop obesity, ~1,500 will develop hypertension, and ~500 will develop hypercholesterolemia.

Table. Power estimates for an identification of an association between environmental exposure and incident diseases in the HELIOS study of 10,000 participants followed up for 10 years. P(E)= prevalence of environmental exposure; RR= relative risk; T2D= Type 2 Diabetes

P(E)	RR	N=500 (hypercholesterolemia)	N=1000 (T2D, Obesity)	N=1500 (hypertension)
		Statistical power (%)	Statistical power (%)	Statistical power (%)
0.10	1.2	23.8	40.4	52.5
	1.3	44.7	71.2	84.3
	1.4	66.5	90.8	97.2
	1.5	83.3	98.1	99.7
0.20	1.2	37.4	61.9	75.9
	1.3	66.7	91.0	97.3
	1.4	87.4	99.0	99.9
	1.5	96.6	99.9	99.9
0.30	1.2	45.8	72.6	85.5
	1.3	76.8	96.0	99.2
	1.4	93.5	99.8	99.9
	1.5	98.8	99.9	99.9

For genetic association studies, we have > 80% power to identify an odds ratio > 1.2 for an association between a Single Nucleotide Polymorphism (SNP) with minor allele frequency (MAF) of > 10% and a

disease with 2,000 prevalent cases amongst the 10,000 population at p=0.05, as the following table illustrates.

At p= 5 x 10^{-8}, we have approximately 80% power to identify an odds ratio > 1.3 for a genetic association between a SNP with MAF of 20% and a disease with 2,000 prevalent cases amongst the 10,000 population.

Table. Power estimates for a genetic association study of 2,000 cases out of 10,000 populations for a range of MAFs and Odd Ratios (OR), in replication testing (p= 0.05) or in genome-wide association (p= 5 x 10^{-8})

MAF	OR	Statistical power in replication testing (%)	Statistical power in genome-wide association (%)
0.10	1.2	89.8	1.3
	1.3	99.7	23.7
	1.4	99.9	76.5
	1.5	99.9	98.2
0.20	1.2	98.9	11.6
	1.3	99.9	77.4
	1.4	99.9	99.5
	1.5	99.9	99.9
0.30	1.2	99.8	26.4
	1.3	99.9	93.8
	1.4	99.9	99.9
	1.5	99.9	99.9

3.3.5 ***Recruitment strategy***

The main phase of the HELIOS study will include up to 10,000 participants. The selection criteria will be Singapore citizens and Permanent Residents aged 30-84 years. The target ethnicity proportion is about 60% Chinese: 20% Malay: 20% Indians and target gender ratio of male to female is 1:1. Initial recruitment methods will include invitation of: 1) Coronary Risk Screening participants from the National Healthcare Group polyclinics at Ang Mo Kio, Hougang and Toa Payoh, 2) residential and community groups, 3) occupational groups (e.g. National Healthcare Group employees), and 4) by word of mouth. Recruitment strategies will be reviewed systematically to evaluate participation, demographic characteristics, responder bias, and costs. Additional recruitment methods may be introduced as necessary.

We also plan a set of pre-pilot, pilot and FFQ validation studies to determine the feasibility and applicability of the protocols, study processes and recruitment strategies. We will include up to 500 participants in the pre-pilot, pilot and FFQ validation phases. Participants in the pre-pilot and pilot phases will be recruited from amongst the same target population as the main study. Potential participants will be invited to take part in either: i. the pre-pilot and pilot phases or ii. The FFQ validation study or iii. Both components if participant is agreeable.

There will be dedicated staff to undertake and manage recruitment of participants to the study. We will establish a single contact point for communication between the study team and participants, enabling engagement with potential participants to be undertaken in a professional and timely manner. Enquiries will range from arranging/ changing an appointment, to discussion of the protocol and dealing with any participants' concerns.

Participants will be offered a modest token of appreciation for their participation in the study. Participants may also be offered low-value HELIOS study promotional materials such as pens, post-it notes and other items of stationery.

3.4 **Characterisation of participants**

Participants will be characterised by questionnaires, physical measurements and biological sample collection, as described in details below. With such extensive assessments, we note that there may be time where for practical/ operational reasons, or due to unforeseen circumstances (for example, staff absence, machine breakdown) it may not be possible to perform all the measurements described during the course of a single visit. In such circumstance and if participants are willing, they may be invited to re-attend on a separate occasion to complete the evaluation. In this circumstance, participants will of course be compensated for the additional inconvenience. Study PIs would set a guideline and determine additional token of appreciation on a case per case basis. This operational flexibility would ensure that participants are given the opportunity to fully experience the study and that missing research data are minimised.

3.4.1 ***Questionnaires***

3.4.1.1 Rationale for computerised baseline questionnaires (estimated time 60 minutes)

A combination of self-administered and nurse-administered questionnaires will be used. A comprehensive examination of risk factors for chronic disease in the cohort will require both the assessment of measured clinical characteristics and other health and lifestyle factors that are difficult to measure objectively, including history of cigarette smoking, alcohol consumption, habitual physical activity, diet, and health history[8-10]. These factors will need to be analysed among the cohort both as main exposures and as potential confounders, as appropriate. Health and lifestyle characteristics are commonly assessed through individually completed questionnaires. Computerized questionnaires have been implemented in other large cohort studies and offer the advantages of efficient data collection and capture, while enabling control for data quality (e.g. missing data are flagged immediately). The set-up and testing phase of the cohort will be used to assess the feasibility and acceptability of the computerised questionnaire-based assessment of factors described below. Where participants are unable to complete the computerised questionnaire, an adult accompanying person or a trained staff member will assist in the completion of the questionnaire. Participants remain eligible to participate in other study stations as long as there are no contraindications.

3.4.1.2 Demographics and socioeconomic status

Age, ethnicity, and socioeconomic status are recognized risk factors for chronic health conditions in Singapore. For example, the risk of myocardial infarction among Indian Singaporeans is more than twofold higher than among Chinese Singaporeans[8], and there is evidence that complications from diabetes[12], depression[13], cognitive function[14], and prevalence of obesity among women[15] vary by socioeconomic status in Singapore. In addition to standard socioeconomic indicators such as income and education, the questionnaire will address characteristics specific to wealth distribution in Singapore, such as vehicle ownership and housing tenure within the context of widespread government-built accommodation.

Appropriate categories of socioeconomic indicators will be identified from national demographic reports and publications.

3.4.1.3 Physical activity

There is convincing evidence that regular physical activity is associated with reduced risks of cardiovascular disease, diabetes, cancer, hypertension, and premature death[16]. In addition, studies of sedentary behaviour (indicated by e.g. screen viewing time or time spent sitting) suggest that inactivity is independently associated with higher risk of fatal and non-fatal cardiovascular disease, type-2 diabetes, and metabolic syndrome[17].

In the most recent national health survey, 39.1% of Singaporeans (18–69 years old) were classified as physically inactive[18]. Physical activity is a modifiable behaviour; as such, the design of public health initiatives could be informed by understanding the nature of the association between different domains of activity and disease risk. Collection of validated questionnaire data on physical activity and sedentary behaviours in the cohort, will allow the relationships between physical activity, inactivity, and the risk of disease to be examined. The self-reported physical activity data will be augmented by objective accelerometer measures.

3.4.1.4 Tobacco smoking

Tobacco smoking is one of the leading preventable causes of morbidity and mortality throughout the world, associated with increased risk of cancer, chronic lung diseases, coronary heart and cardiovascular diseases and other unfavourable health outcomes. The age-standardized prevalence of smoking in Singapore was estimated in 2011 at 24% among men and 4% women[19]. Although there is evidence of a decline in smoking prevalence among men[18], the healthcare costs associated with treatment of tobacco-related conditions in Singapore remain considerable; it is estimated that COPD alone costs 9.9 million US dollars per year[20]. Current and past use of tobacco will be assessed thoroughly, drawing on comprehensive questions from the European Prospective Investigation into Cancer, the UK Biobank, and other studies.

3.4.1.5 Alcohol drinking

In the 2010 Singapore National Health Survey, 42% of respondents reported no alcohol consumption and an equal proportion reported consuming alcohol less than 3 times per month; these estimates indicate on average a low per capita consumption recorded from 1960s onward[19]. However, among drinkers, there is evidence that binge drinking has increased, particularly among young adults[21]. There is convincing evidence that alcohol is a major cause of cancers of the upper gastrointestinal tract and is associated with increased risk of cancers of the liver, colorectum and breast[22]. In contrast, moderate alcohol intake appears to be protective against cardiovascular disease[23]. Accordingly, assessment of alcohol as a main exposure and as a potential confounder is essential in studies of chronic disease risk. The cohort questionnaire will assess current and historical alcohol intake.

3.4.1.6 Diet

Diet is a modifiable lifestyle factor associated with the risk of cardio-metabolic disease and cancer in the form of possible protective factors (including vegetables, nuts, and Mediterranean-style dietary patterns) and potentially harmful factors (including red and processed meats, trans–fatty acids and high glycaemic index/load foods)[24;25]. Similarly, prevention and treatment of diabetes has been favourably associated

with dietary patterns characterised as low-carbohydrate, low-glycaemic index, Mediterranean-style, or high-protein[26;27]. Knowledge of food, nutrients, and dietary patterns in relation to chronic disease prevalence and incidence could be used to inform the development of prevention and management strategies for chronic diseases in Singapore. In particular, the use of a standardised approach to assess diet-disease associations among Singaporeans of Chinese, Malay and Indian origin may provide valuable insight for the development of interventions, given the potential for ethnic variability in dietary patterns and other lifestyle factors. In the cohort, assessment of usual intake via detailed questionnaires can potentially be enhanced by the analysis of nutritional biomarkers in the blood and urine samples collected at baseline.

3.4.1.7 Mobile phone use

Mobile phone use in Singapore is widespread yet the long-term health effects of mobile phone use are unknown. A meta-analysis of the risk of brain tumours related to mobile phone use yielded null results, but it has been noted that there is considerable methodological heterogeneity between studies and a paucity of data on long-term outcomes[28;29]. A recent report from the International Agency for Research on Cancer (IARC) classified mobile phone use and other radiofrequency electromagnetic fields as a possible carcinogen (group 2B) based mainly on assessment of risk from case-control studies[30]. The World Health Organisation (WHO) has indicated that long-term cohort studies of mobile phone use and health are a high priority for research. To the best of our knowledge, assessment of mobile phone use has not been included in Singapore cohort studies. The use of standardized questions on mobile phone use will enable examination of current and past use of mobile phones in the cohort, and allow for participation in consortia of possible health effects of mobile phone use.

3.4.1.8 Sleep

The questionnaire will assess duration of sleep and usual sleep patterns, as sleep deprivation and disruption of sleep patterns are risk factors for the development of type-2 diabetes[31], cardiovascular disease[32] and other health conditions such as hypertension and hypercholesterolemia[33;34].

3.4.1.9 Mood

There is epidemiologic evidence of a link between depression and type-2 diabetes mellitus[35;36]. Depression is associated with a 60% higher risk of incident type-2 diabetes, whereas prevalent type-2 diabetes is only modestly associated with greater risk of depression[36]. The cohort is an ideal population for investigating this relationship due to the high prevalence of diabetes in Singapore and the prospective nature of the proposed study. Accordingly, validated instruments for assessment of depression and anxiety in Asian populations will be included in the baseline questionnaire.

In addition, cognitive function will be assessed using a standardized method described in the following section on physical and clinical measurements.

3.4.1.10 Childhood and early life history

A number of childhood health exposures will be assessed by the cohort questionnaire, including maternal and paternal smoking during pregnancy, second-hand smoke exposure, and breastfeeding. There is evidence that maternal smoking is an independent risk factor for increased body mass index and waist circumference in adulthood[37]. In addition, exposure to second-hand tobacco smoke in children is associated with health conditions including asthma and cardiovascular effects and is also related to a

higher likelihood of smoking initiation[38]. Conversely, observational studies suggest that individuals who have been breast-fed have lower levels of blood total cholesterol, lower risk of type-2 diabetes and marginally lower levels of adiposity and blood pressure in adult life[39].

3.4.1.11 Personal health

Information on indicators of health status and disease history (including family history and medication use) are required for analysis of potential predictors of future disease (both as main effects and as potential confounders), to describe prevalent health states, and to identify subgroups in sensitivity analyses.

Aspects of personal health to be addressed by the questionnaire include: self-rated health; vision; hearing; oral health; weight change throughout adulthood; chest pain (Rose's angina screening tool); sexual activity and reproductive health (menstruation, fertility, fecundity, use of oral contraceptives and menopausal hormone therapy); diagnosis of common health conditions (age at diagnosis, treatment type); asthma (the Asthma Screening Questionnaire) and skin health.

3.4.1.12 Reproductive history

Reproductive history among women is a recognized risk factor for cardiovascular disease and cancer[40;41], therefore information on menstruation, birth control, fertility, pregnancy, parity, obstetric outcomes, breastfeeding, and menopause and hormonal therapy will be collected.

3.4.1.13 Cognitive function (estimated time 15 minutes)

Cognitive function is affected by ageing and certain diseases, such as Alzheimer's disease and Parkinson's disease, and cognitive decline is becoming one of the leading causes of disability globally. Reduced cognitive function may also be associated with general vascular risk factors[42], physical inactivity[43], smoking[44], diabetes[45], obesity and hypertension[46]. Brief self-assessed tests of cognition are used in the UK Biobank, Airwave, and Qatar Biobank studies. The assessment consists of paired-associated learning tests to assess global cognition and reaction time tests for touch screen administration[47].

Cognitive function will be assessed either using software previously used in the Airwave and UK Biobank studies or a locally developed version.

3.4.1.14 Medications and health supplements

Current use of mediations will be recorded. Use of dietary supplements and traditional medicines will also be assessed, both as source of nutrient intake and as a marker of health-conscious behaviour[48]. A brief nurse-administered questionnaire will address details of occupational history, past surgery, medical conditions, and medication use, as these questions require lengthy and complex response structures that require expertise to navigate efficiently (e.g. SNOMED for medical conditions and surgery).

3.4.2 ***Clinical/physical measurements***

The range of clinical and physical measurements is selected to cover diverse disease risk factors and phenotypes. For each clinical and physical measurement we have outlined the scientific rationale for its inclusion and the estimated time taken to collect each measurement.

All clinical and physical measurements will be undertaken by nurses or technicians, trained and accredited in each of the specific techniques, using written internationally accepted protocols, and overseen by Clinician Scientists with expertise from the relevant disciplines. An ongoing system of performance monitoring and quality control assessment will be put into place.

3.4.2.1 Anthropometry (estimated time 8 minutes)

Standing height and body weight are both predictive of disease risk and mortality and are used to calculate the body mass index (BMI). BMI is a marker of overall body fat and is used to define overweight and obesity, which are well established, strong risk factors for cardiovascular disease (CVD), stroke, high blood pressure, and certain cancers as well as other conditions such as osteoarthritis[49-54]. Anthropometric measures such as waist, hip, and chest circumference, and leg length, are measures of regional body fat and muscle distribution. While these measures may be correlated with BMI, they also show independent associations with disease risk. Abdominal obesity is closely associated with future risk of several chronic diseases, and large studies have suggested that as indicators of abdominal obesity, waist circumference or the waist-to-hip ratio may be better predictors of the risk of future disease than BMI[49]. Both measures have also shown associations with diabetes mellitus, metabolic syndrome, cardiovascular disease, and certain cancers[50;55-58].

Body weight and height will be measured once wearing light clothing using computerized measuring instruments with automated data capture. For waist, hip, and chest circumference, and leg length (inseam) measurements, a non-stretchable sprung measuring tape will be used. Chest circumference will be measured at the largest circumference of the chest, typically just above the nipple line. Waist circumference will be measured at the mid-point between the iliac crest and the lowest rib, at the smallest circumference of the natural waist (if visible), and hip circumference will be measured at the maximum circumference of the buttocks, over the greater trochanters. The readings of chest, waist, hip circumference, and leg length measurements will be manually entered in the IT system which will automatically highlight impossible or implausible values.

Modern weighing machines also offer the opportunity to measure body impedance at the same time as measuring weight by passing a very weak, imperceptible electric current between limb leads. Using a computer algorithm including variables such as sex, weight, age and height, an estimate of body fat and fat free mass can be obtained. Information on percentage of body fat would enable further exploration of the associations between BMI and waist circumference with total body fat and examine whether total body fat is superior to either BMI or waist circumference in determining overweight and obesity and therefore in predicting future disease risk. Body fat composition by bioimpedance will be measured using an Inbody 770 device or equivalent.

Figure 1 ***InBody 770 body composition***

3.4.2.2 *Dermatology Exam (estimated time 7 minutes)*

The dermatology exam includes i) skin physiology measurements of trans-epidermal water loss (TEWL), skin surface pH and skin surface moisture; ii) a skin examination; and iii) skin microbiome sampling. The skin physiology measurement component consists of measuring TEWL by a vapometer, surface hydration by a MoistureMeter SC and skin surface pH by a pH meter. Each skin physiology parameter comprises 3 readings of one of the inner forearms to get an average reading. The targeted skin examination involves screening by a technologist of the following selected parts of the body for presence of visible flexural dermatitis or psoriasis skin plaques. Sites include front of elbows, behind the knees, front of ankles, around the neck and around the eyes for visible flexural dermatitis. Sites include elbows, knees and shins, lower back, hairline (anterior, behind ears posterior) and nails for presence of psoriasis. Details of the skin microbiome sampling component are presented in the 'Biological Sampling' section of the protocol.

3.4.2.3 *Blood pressure (estimated time 4 minutes)*

Elevated blood pressure is an established risk factor for coronary heart disease, congestive heart failure, and the major modifiable risk factor for stroke[59]. The association between blood pressure and cardiovascular diseases is continuous and graded; among middle-aged individuals, a 20 mm Hg difference of usual systolic blood pressure (or, approximately equivalently, 10 mm Hg usual diastolic blood pressure) is associated with more than a two-fold difference in the stroke death rate, and with two-fold differences in the death rates from coronary heart disease and from other vascular causes[60]. In addition, higher blood pressure has been associated with increased risks of dementia[61;62], cognitive function decline[63;64], and type-2 diabetes[65].

Blood pressure will be measured 3 times in the right arm using an Omron (or equivalent) blood pressure monitor and results will be imported electronically into the study database.

3.4.2.4 Lung function - spirometry (estimated time 5minutes)

Spirometry is one of the most widely used techniques to assess lung function, which is a strong predictor of all-cause mortality, chronic lung disease, lung cancer, and cardiovascular disease[66-70]. Spirometry is dependent on maximal effort by the participant, and includes measurement of the forced expiratory volume in 1 second (FEV1) and forced vital capacity (FVC). Information on lung function will allow investigation both of prediction of future disease and the different aetiological pathways linking lung function with other chronic diseases.

Lung function will be measured using the MIR Spirolab monitor (or equivalent) and results will be imported electronically into the study database. Participants will be asked to provide up to 4 recordings to overcome the learning effect.

3.4.2.5 Electrocardiogram (ECG) (estimated time 5 minutes)

The electrocardiogram (ECG) is the most widely used non-invasive test for the diagnosis of coronary heart disease. The standard 12-lead ECG provides a summary report of the electrical activity of the heart and allows the identification of a wide variety of cardiac abnormalities, such as "silent" myocardial infarction, other evidence of ischaemia, conduction defects, ventricular hypertrophy, and arrhythmias.

ECGs will be recorded using a GE Healthcare CASE device (or equivalent) according to published international standards [69] and results will be imported electronically into the study database.

3.4.2.6 Arterial stiffness (estimated time 10 minutes for three measurements per participant)

Arterial stiffness is determined by structural and functional components related to the intrinsic elastic properties of the artery. Any decrease in conduit vessel elasticity, or an increase of arterial stiffness, is a hallmark of arterial ageing and has unfavourable cardiovascular consequences. Pulse wave velocity (PWV) is a measure of arterial stiffness, and higher PWV has been consistently linked with greater risks of cardiovascular disease events. A 2010 meta-analysis of 17 cohort studies reported that a 1-m/s increment in aortic PWV was associated with 14% higher risks of total cardiovascular disease events[71]. Although arterial stiffness has been measured in several large-scale cardiovascular disease cohorts, collection of such data in the cohort will allow assessing prediction of future cardiovascular disease and its inter-correlations with other measures of sub-clinical atherosclerotic disease.

We will use the VICORDER, a non-invasive device that allows pulse wave velocity to be measured simply and rapidly, or equivalent. Participants will be asked to recline at a 30^{o} angle on a couch, blood pressure cuffs are placed over the brachial artery on the right upper arm and over the femoral artery on the right thigh. Pressure within the cuffs is increased and fluctuations around this pressure are then used to determine a pulse volume waveform that is closely related to intra-arterial pressure. VICORDER has already been used successfully in other large scale epidemiological studies such as the Airwave study. The technique is simple to administer and well tolerated.

3.4.2.7 *3-D carotid ultrasound (estimated time 10 minutes)*

Atherosclerosis, the main underlying cause of cardiovascular diseases, remains sub-clinical for extended periods of life. This sub-clinical phase of atherosclerosis is thought to be characterised by thickening of the intima media thickness (IMT) and formation of atherosclerotic plaques in the carotid arteries; IMT and carotid plaques are considered markers of early atherosclerosis, its anatomic extent and progression. People in the top quintile for carotid IMT have been shown to have three-fold higher risks of coronary events, strokes or vascular deaths compared with those in the lowest quintile after adjustment for traditional risk factors[72]. Recently, 3-D carotid ultrasound has become available which also provides information on carotid plaque area and volume (Figure 2); both phenotypes have been associated with increased risk of ischaemic stroke and coronary heart disease[73]. Total plaque area has also shown strong associations with stroke, vascular death or myocardial infarction[73;74]. IMT and plaques are considered separate phenotypes as they are biologically and genetically diverse[74] and they may be associated differentially with cardiovascular risk factors.

Carotid IMT has been examined in many large epidemiological studies; however, other parameters of carotid plaques have only been assessed in a few small-scale cohort studies to date. The present study will provide a more comprehensive evaluation of their potential use as a marker of subclinical atherosclerosis and for cardiovascular disease risk prediction.

3-D carotid ultrasound scans will be performed on both left and right carotid arteries with participant recumbent at 45 degrees using a Philips EPIQ 7 (or equivalent) device. The test is non-invasive and well tolerated.

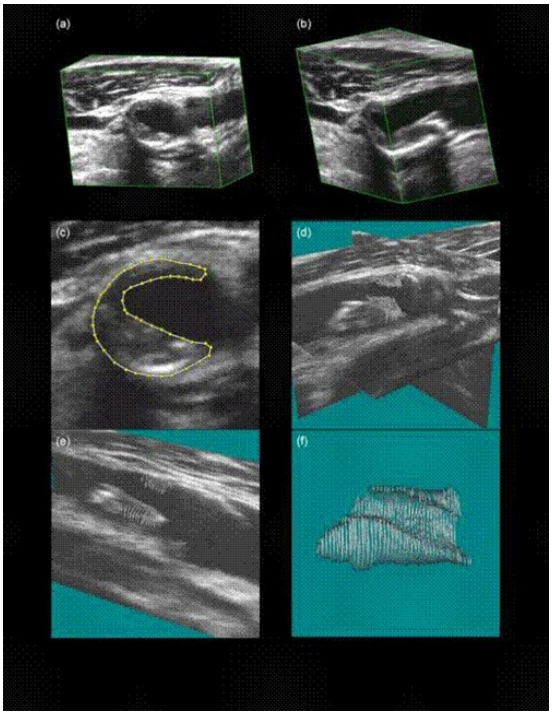

Figure 2 **An example of plaque volume measurement using 3-D carotid ultrasound** *(http://www.imaging.robarts.ca/SPARC/3-D-Plaque-Measurement)*

3.4.2.8 Ophthalmology assessment (estimated time 15 minutes)

Retinal vascular characteristics have been associated with hypertension, stroke, diabetes, and ischaemic heart disease even after adjustment for conventional risk factors[75-77]. Digital colour photographs of the retina will allow the assessment of age related macular degeneration, an important cause of vision loss. Digital colour photographs of the retina will also provide information on retinopathy and vascular geometry.

Optical coherence tomography angiography (OCTA), optical coherence tomography (OCT), and colour fundus photography will be used in the ocular imaging protocol.

OCTA is a new imaging technology that is able to produce high-resolution images of the vasculature within various layers of the retina and choriocapillaries. The current gold standard for visualization of the retinal vasculature is fluorescein angiography, which is invasive and requires intravenous injection of contrast. OCTA, on the other hand, is non-contact and non-invasive and does not require intravenous access, thus reducing inconvenience and avoiding the risk of allergic reactions or anaphylaxis. It is also less costly than conventional fluorescein angiography.

OCT is also a non-contact and non-invasive imaging technique that can produce high resolution cross-sectional images of the retina, the choroid, retinal nerve fibre layer and optic nerve head. OCT plays an essential role in the evaluation and management of ocular diseases. OCT can show structural changes in the retina, which may be related to ageing or a subclinical indication of the onset of specific diseases. For example, the choroid, which is a vascular layer adjacent to the retina, plays specific roles in the normal physiology of the eye and in retinal diseases. It may also be a marker for systemic diseases, such as hypertension or other aging-related degenerative conditions.

A colour fundus photograph provides an en-face view of the retina, and allows detection of abnormalities of the retina, retinal vessels and optic disc. It provides an objective record of the fundus features, and can be correlated with both OCTA and OCT scans.

In addition, visual acuity, refraction, intra-ocular pressure, and corneal biochemical properties will be assessed during the baseline recruitment visit. All measurements will be made with the participant in a sitting position in a darkened room, according to internationally recognised protocols. Contact lenses will be removed, where appropriate.

For retinal imaging, mydriasis will be induced about half an hour before the assessment. We will apply 1-2 drops of Guttate Tetracaine, followed by 1-2 drops of 1% topical Tropicamide to both eyes to achieve mydriasis. This is a standard practice for the majority of eye examinations and eye studies, and is very commonly performed. This will be administered by the trained members of the study team. Mydriasis may cause blurring of vision for a period of 4 to 8 hours and induce photophobia, lack of accommodation, glare, and decreased contrast threshold and high-contrast visual acuity. However, most patients find these relatively mild and the side effects will gradually disappear over 4 to 6 hours. The risk of precipitating angle closure glaucoma has been found to be very low (1 to 6 in 20,000)[78]. To further lower this risk, the participants will be screened for features that predict acute angle closure, such as eclipse sign at the nasal side of the anterior chamber after the eyes are shined with a penlight.

The contraindication for the eye assessments, including mydriasis, will comprise:

1. Have ever had a detached retina
2. Have had any eye surgery within the last 4 weeks
3. Have had any other surgery in the last month (apart from skin)
4. Have had active eye infection (redness, swelling, pain, discomfort, discharge)
5. Presence of prosthetic eyes
6. For retinal scan only, photophobia, recent or new onset blurring of vision, or double vision
7. For pupil dilation, any allergies to Tetracaine or Tropicamide

3.4.2.9 Physical fitness test (estimated time 15 minutes)

Physical activity and cardiorespiratory fitness are two correlated but distinct phenotypes which are associated with improved health and quality of life. Cardiorespiratory fitness has been measured in only a few epidemiological studies; nonetheless, low fitness has been associated with increased all-cause and cardiovascular disease mortality risks[79;80]. It is still unclear whether physical fitness provides a more accurate measurement of physical activity levels than a physical activity questionnaire, or whether physical fitness influences risk of disease through aetiological pathways independent of physical activity. The availability of information on both physical activity and fitness in the study will allow this hypothesis to be tested and examine the independent effect of each of these phenotypes on disease risk.

We will use a sub-maximal treadmill test to evaluate physical fitness. The protocol for the fitness test modified based on an existing protocol used in another LKC Medicine study (NTU IRB-2015-05-029) with a proven track record of safety and acceptability. The test will comprise a walk at speed of 4km/h, 5km/h and 6 km/h for 2 minutes each, after which, there will be a 3%, followed by a 6% increase in gradient at 6km/h for 2 minutes each. Heart rate will be monitored throughout the test. Blood pressure will be measured at the beginning and end of the treadmill test. During the exercise test, a continuous 3-lead ECG may be captured for non-diagnostic purposes to provide additional cardiovascular phenotype (e.g. heart rate variability). The test will be terminated early if 80% of age-predicted maximum heart rate is achieved, or at participant request.

Exclusion criteria for the fitness test will be those with:

- **Pregnant** (self-reported or positive pregnancy test)
- **Uncontrolled diabetes** (blood glucose <3.9mmol/L or >15mmol/L). Blood glucose will be measured by glucometer on all patients with known diabetes, at the treadmill station before the treadmill exercise test is started:
    - If blood glucose is <3.9mmol/L, the participant will be given some light refreshments (3 tsp of table sugar or honey dissolved in water, or half a can of regular soft drink equivalent to 15g of simple carbohydrate). Blood glucose will be re-checked 15 minutes later and if blood glucose is still <3.9mmol/L, participants will be excluded from the test and advised to see GP for early review.

  - If blood glucose is >15mmol/L, the treadmill test will not be performed and will follow the protocol for the management of abnormal results.
- **Uncontrolled high blood pressure** (BP >160/100mmHg)
- **Symptoms of chest pain or breathlessness on exertion**.
- **Medical conditions or disabilities that are a contraindication to exercise,** for example recent myocardial infarction, recent major surgery, musculoskeletal defects, etc .

Participants may elect not to attempt the physical fitness test. In the event that the participant becomes symptomatically unwell, the treadmill test will be stopped and the participant will be taken to rest on a couch. A full set of vital observations will be taken (heart rate, blood pressure, oxygen saturation, blood sugar), a 12 lead ECG recorded, and the participant connected to ECG monitoring. The HELIOS medical support will be contacted to review the participant and to provide further advice. In the highly unlikely event of a cardiac arrest, clinical staff members are BCLS/CPR-AED trained, and an automated defibrillator is available.

3.4.2.10 Physical activity monitoring using an accelerometer (estimated time 7 days)

Historically, questionnaires have been used to quantify physical activity in large-scale population studies. Although questionnaires do provide standardised data that can be compared with international studies, questionnaire data show modest reproducibility and limited validity compared to gold standard assessments of energy expenditure. More recently, development of triaxial accelerometers has provided cost effective and reproducible measurement of physical activity in free living humans. Accelerometers have been successfully used among participants in recent large-scale population studies, including the UK Biobank, the German National Cohort, and NHANES study. Modern accelerometer devices are small and lightweight, worn on the wrist or waist, and can distinguish between different types of activity (e.g. exercise and sedentary activities). Measurements of physical activity by accelerometer correlate very closely (r>0.8) with measurements of energy expenditure by doubly labelled water (the gold standard).

All participants will be asked to wear wrist or waist based accelerometer devices for 7-days, and provided with pre-paid envelopes to return the devices.

3.4.2.11 Hand grip strength (estimated time 3 minutes)

Total body muscle strength declines with age, and is a major predictor of morbidity, loss of function, and mortality in later life. Grip strength is a simple method of measuring muscle strength. Low grip strength has been associated with falls, disability, impaired quality of life, and higher mortality[81-83]. In a recent meta-analysis, people in the lowest quarter of grip strength had 67% higher risk of all-cause mortality when compared with those in the highest quarter[81].

Right and left hand grip strengths will be measured using a Jamar Plus Hand Dynamometer (or equivalent) (Figure 3). Contraindications include severe joint conditions in the wrists or fingers.

Figure 3A Jamar Plus Hand Dynamometer

3.4.2.12 Dual energy X-ray absorptiometry (DXA) scan - bone health and body composition (estimated time 15 minutes)

Dual energy X-ray absorptiometry (DXA) is the gold standard for the measurement of bone mineral density and is widely used in research and clinical settings. Participants lie on their backs on an X-ray table to undergo a body scan (Figure 4). The technique can measure any body site, but lumbar spine and hip are most commonly measured regions, while whole body scans may also provide information on body composition.

Osteoporosis is a skeletal disease characterized by low bone mass. It is a major public health problem with high prevalence; about 50% of women and 20% of men over 50 years old will have a fragility fracture in their remaining lifetime[84]. Many epidemiological studies have investigated the lumbar or hip measurements of bone mineral density via DXA as a predictor of subsequent events. Overall, the risk of osteoporotic fracture is increased two-fold for every standard deviation (SD) decrease in bone mineral density with the risk being continuous and graded over the range of bone mineral density[85].

Measurement of bone mineral density in the study will enable the examination of the prediction of this measurement for future outcomes but also serve as a baseline phenotype for early cross-sectional analyses. It would also be useful for the determination of reference values of bone mineral density among the different ethnic groups of the Singaporean population. Moreover, new high resolution DXA instruments have good image quality, which allow grading of osteoarthritic changes, such as joint space narrowing, osteophyte formation, subchondral sclerosis and cysts, at the hip and knee. Therefore, the DXA scan will not only provide information on bone density but also on osteoarthritic changes.

Whole body DXA provides a measurement of total and proportionate bone, fat, and lean mass, and also gives the distribution between segments of the body (arms, legs, head, thorax, trunk, and pelvis) automatically within seconds of the scan. This information will complement the standard anthropometric measurements collected and will give a more accurate measurement of body fat and its regional distribution. It has been recognized that fat distribution is an important characteristic in the metabolic and clinical alterations associated with adiposity. The diverse measurements of body fat and its regional

distribution would allow the examination of the relative value of each measurement in relation to cardio-metabolic and other conditions.

The test involves the participant lying supine on the scanner for the whole body scan and hip, while for the lumbar spine scan, the hips and knees are flexed and supported with a cushion. The test involves a low-dose X-ray (Hologic Horizon W model or equivalent), equivalent to 3 hours of background radiation or ½ a chest X-ray.

This amount of radiation exposure is considered safe. The equipment will be operated by trained and qualified clinical research staff, working under the supervision of the Radiology Department and the Radiation Protection Advisors at TTSH, NHG.

Figure 4 ***A dual energy X-ray absorptiometry (DXA) couch***

3.4.2.13 Audiometry and tympanometry hearing examinations (estimated time 15 minutes)

Cochlear is the sense organ that translates sound into nerve impulses to be sent to the brain. Changes to the cochlear is a hallmark of age-related hearing impairment. Cochlear microcirculation characteristics have been associated with diabetes, central adiposity, cardiovascular diseases, cognitive decline and dementia[86-90]. The hearing examination is a three-part procedure which includes 1) otoscopy; ii) tympanometry; and iii) pure tone air conduction and bone conduction audiometry.

Otoscopy is the visual examination of the outer ear - including the auricle, ear canal, and eardrum using an otoscope. Otoscopy has two purposes: 1) to identify abnormalities that may require alternate audiometric procedures or influence the results obtained; and 2) to identify conditions that may require medical referral. An otoscope is a small, handheld instrument with a light that is directed through a funnel-like tip to illuminate the ear canal for examination.

Tympanometry is an objective test of middle ear function. Tympanometry tests the mobility of the eardrum, from which information regarding the function of the middle ear system can be inferred. The test is conducted by sealing off the entrance to the ear canal with a rubber cuff, changing the air pressure within the ear canal, and recording the flexibility of the eardrum in response to the changing pressure.

The test is objective in that the examinee is completely passive to the test process. The tympanometer is automated and performs the test and records the results without any need for response or feedback from the examinee. Tympanometry can point out problems with how the ear is function in; which may impact hearing sensitivity, but it does not directly indicate how well a person can hear.

Audiometry is the measurement of hearing sensitivity. Pure tone air and bone conduction audiometry, which tests the hearing sensitivity of the entire auditory system by presenting pure tone signals generated by a diagnostic audiometer to the ear through earphones or bone vibrator and varying the intensity of the signals until the level is identified at which the person is just able to hear the sound. This level is known as the person's threshold. Clinically, threshold is usually defined as the level at which the subject will be able to detect the signal 50% of the times that it is presented. Pure tones are presented at frequencies across the range of human hearing. Because the tones are presented at the external ear or through the mastoid, and processing of those signals through the auditory nervous system is necessary in order for the subject to be aware and respond that the signal was heard, this type of testing evaluates the auditory system as a whole and is capable of identifying hearing problems at almost any level within the auditory system.

As per standard clinical practice, subject will be tested twice per ear with tympanometry, followed by once with pure-tone audiometry. All measurements will be performed by a trained audiologist in a sound-proof booth according to internationally recognized protocols.

3.4.2.14 <u>3D-Infrared imaging for quantification of body morphometry (estimated time 10 minutes)</u>

Body shape is well recognised to be an accurate surrogate marker of body composition and can be used to rapidly distinguish lean from obese, as well as gynoid versus android patterns of obesity. These observations provide the opportunity to use infrared imaging and machine learning for quantification of body shape and rapid assessment of body composition without the need for specialised equipment. To advance this hypothesis, we will take 3D-infrared imaging of participants. The images collected will be used to create a database of human body shapes, and to quantify the relationships of body morphometry with body composition determined by DXA and other established measurement techniques. Body images will be taken fully clothed. No facial scan will be taken. The images from this exercise will be stored securely at NTU LKC Medicine in the HELIOS Study database.

3.4.3 ***Biological Samples***

We will collect, process and store biological samples including blood, urine, saliva, stool, and skin tapes. The aim is to collect informative biological material from participants, in such a way that it is of maximum utility to a broad range of researchers and protected from degradation, whilst in storage.

At this stage, we envisage carrying out a wide range of molecular profiling approaches on the stored samples. This includes, but is not limited to, genome sequencing, epigenetic profiling, quantification of gene transcription, metabolomic and proteomic profiling and microbiome assessments. This may be applied to all the biological samples collected. Since technology and analysis tools are developing continuously, this not an exhaustive list of possible future research based on the biological samples. The informed consent provided by the participants at entry to the study makes explicit reference to the authorisation to conduct a broad range of health-related research based on both data and stored biological samples in keeping with the general aims of the HELIOS Study. Therefore, there is no need to re-contact the participants when specific research projects involving data and biological samples are planned.

At enrolment to the HELIOS study, we will measure a panel of biomarkers on the baseline blood samples representing clinically relevant parameters for health screening. We initially propose:

- Full blood count
- Urea and renal function tests
- Lipid profile
- Glucose and HbA1c

This list may be updated as the study proceeds.

3.4.3.1 Biological Sample Collection

The aim is to collect samples that would allow the widest possible range of assays that could plausibly be envisaged for the future studies. Each sample tube has a unique barcode. The details of biological sample processing are available in the working protocol and will be continually assessed.

3.4.3.1.1 Blood Collection

A total of up to 70ml of blood will be collected from each participant at a single time point by a certified phlebotomist during the baseline assessment visit. Blood draw is performed by venepuncture of a superficial arm vein using sterile procedures, when the participant is comfortably seated. The “vacutainer” system will be used to collect these blood samples. During venepuncture, the hypodermic needle is connected to these vacutainer tubes. These tubes contain the required additives. They are held under a slight vacuum, which draws sufficient blood to fill them. A sterile plaster will cover the phlebotomy site after the procedure and the arm will be elevated to ensure that bleeding has stopped.

The risks of taking blood include momentary discomfort at the site of the blood draw, a bruise at the point where the blood is taken, redness and swelling of the vein, infection and a risk of fainting. If the volunteer is lightheaded, he/she will be reclined and monitored until symptoms resolve.

3.4.3.1.2 Urine Collection

A morning sample of urine is the specimen of choice. The participant has to void the first portion of the urine stream into the toilet. The urine midstream is then collected into a clean sample container, properly capped and placed into a provided zip-lock biohazard bag. There are no risks to participants in providing urine samples.

3.4.3.1.3 Saliva Collection

The participant has to refrain from eating, drinking, or oral hygiene procedures for at least 1 hour prior to the collection. The participant is given distilled drinking water, asked to rinse their mouth out well. Five minutes after this oral rinse, the participant is asked to spit into a 20 ml sterile tube. There are no risks to participants in providing saliva samples.

3.4.3.1.4 Stool Collection

The participant has the option to provide or not to provide a stool sample. The willing participant has to pass stool directly into a clean dry container provided. A small spoon attachment on the lid of the specimen container is used to remove a small sample from the middle of the stool. A heaped spoonful of stool is sufficient. The stool sample is placed in a specimen container and the lid is tightly closed. Samples must be kept cold after collection. There are no risks to participants in providing stool samples. For participants who cannot provide stool samples during study visit, we will provide a stool collection kit and an envelop stamps to take home. Participants will need to collect, store, and return the stool samples by mail according to the instructions provided. We have piloted this and observe 100% return rate with approximately 7 days of return since study visit.

3.4.3.1.5 Skin Tape Collection

Skin sample is collected using aseptic technique by mechanical disruption of the skin surface. The participant's skin at the antecubital fossa, upper back and volare forearm is collected by skin stripping method; using Cudem circular tapes by tapping approximately 50 times on the skin surface until the tape losses its stickiness. There will be some redness on the skin surface.

3.4.3.2 Sample Processing and Storage

3.4.3.2.1 Blood samples

Blood samples are processed according to well established protocols; with/without addition of preservatives, with/without centrifugation and subsequent aliquoting into smaller cryovials for long term storage in medical grade freezers. The blood sample preparation, storage and labelling system will comply with international best practice in order to future-proof the samples.

3.4.3.2.2 Urine, saliva and stool samples

The urine, saliva and stool samples are immediately placed on ice, aliquoted within half an hour of collection and stored in -80°C freezers.

3.4.3.2.3 Skin Tapes

Two skin tapes, one each from the antecubital crease will be collected from each participant. These tape specimens do not require immediate processing but will be stored at -80°C as soon as possible but no longer than 30 minutes.

3.4.3.3 Processing Samples and Archiving

Participant's samples will be processed manually, by one or two technicians using a Laboratory Information Management System (LIMS). Biological samples will be prepared and stored in the freezers of Nanyang Technological University Lee Kong Chian School of Medicine.

3.4.4 ***Participant Feedback and Incidental Clinical Findings***

All participants will receive written feedback on clinically relevant results based on their baseline screening data, including biochemical markers, in the form of a structured report, accompanied by a booklet explaining the meaning of the tests and the interpretation of the results. The aim is that this report will be sent to participants within 4 weeks of the appointment date. The report format and content are expected to evolve based upon experience and feedback from participants as the study progresses, subject to continued ethics committee review. Initial expectations are to routinely report back the following test results to all participants as a "HELIOS research health report":

1. Height, weight, waist circumference and body composition e.g. body fat percentage.
2. Blood pressure
3. Electrocardiogram
4. Dual energy X-ray absorptiometry (DXA) scan
5. Full Blood count
6. Lipid profile
7. Glucose and HbA1c
8. Uric acid
9. Active smoking status
10. Self-reported medical history
11. List of medications

In addition, a written protocol will be established to describe what represents a clinically significant abnormality requiring clinical action. Participants with non-urgent clinical findings will receive a report within approximately four weeks to advise them to see their own doctor for further advice. Major clinical findings will be discussed with a senior NHG physician on the same day for a decision on appropriate action. Clinical findings of immediate significance (e.g: evolving myocardial infarction on ECG) will be referred immediately to Accident & Emergency (A&E). If the abnormal clinical findings become evident after the participant has left the screening clinic, all reasonable measures will be taken to contact the participant urgently.

All other measurements conducted in the course of the HELIOS study are being done for **research** and not for clinical purposes, and will not therefore be fed back to participants routinely. However, if the research team do identify an abnormality during the course of the HELIOS assessment, which they believe might have important clinical significance for the participant, this will be reported to the participant. An example of this would be severe carotid stenosis or evidence of significant retinopathy. Since this is not a clinical service, and the data are not systematically reviewed by a clinical team, we cannot guarantee that we will identify all abnormalities from the HELIOS tests that have been done for research purposes.

Since the significance of research findings are typically uncertain, feeding back research findings could lead to inappropriate anxiety or other indirect harm. Therefore, we will not be reporting back the results of any additional tests done on the biological samples in the future (more than 6 months after the HELIOS assessment) and specifically we will not be reporting back the results of genetic evaluation, except in the context of further research sub-studies (that would require separate IRB approval).

3.4.5 *Long-term follow-up of participants*

The cohort design of the HELIOS study will require participants to be followed up for many years starting immediately after baseline to identify people who will develop particular diseases and relate this to the extensive range of risk factors captured at baseline. This presents an invaluable opportunity to investigate the causes and natural history of a wide range of diseases and health conditions that affect the population of Singapore. There are two broad approaches to follow-up that will be adopted:

- Routine record linkage: where routine health records are available, we will endeavour to individually link participants to their health records for a wide range of medical conditions including cancer, hospitalization, and primary care consultations including drug history, results of laboratory investigations and imaging. For participants who die during follow-up we will obtain a copy of the death certificate including specific cause of death.

- We envisage accessing health records of participants held by NHG, Singapore Cancer Registry, and Ministry of Health. We also anticipate linking to the impending centralised electronic medical record system, currently being developed in Singapore (Precision Medicine Project). Since it is not possible to describe the health records that will be available in the future, it is not possible to provide an exhaustive list of data sources that will be used, and we aim to access a wide range of systems (current and future) that hold health data as is usual practice in large scale population studies internationally.

- Re-contact with participants: Direct re-contact with participants provides a valuable means to capture reliable information on a wide range of risk factors and medical conditions. The written consent from all participants at the baseline visit will include permission to re-contact them in future for a re-screening examination or to take part in specific research projects based on genotype and/or phenotype. As used successfully in other large-scale studies (e.g. EPIC), this might involve attending a repeat baseline visit to the research centre. Follow-up using web-based questionnaires may provide a cost-effective option for future contacts.

3.4.6 *Pre-pilot and pilot phases of the HELIOS study*

Prior to any epidemiological study, an evaluation of study feasibility and acceptability is compulsory. Any assessments that have not been previously administered in local population also require validation. For instance, all questionnaires would need to be translated and the quality of translation assessed objectively through a systematic interview. Staff competency measured through intra- and inter-variability of each measurement needs to be evaluated. Device variability needs to be assessed to ensure accuracy. The reproducibility of each measurement also needs to be recorded to ensure a high quality research data. Therefore, the HELIOS study will carry out evaluation of study procedures in pre-pilot phases (limited aspects of the study protocol) and a pilot phase (complete evaluation in approximately 180 people). The protocol for the pre-pilot phase is provided (**Appendix 1 HELIOS study: pre-pilot and pilot phases**). The HELIOS study will also validate a novel, electronic FFQ developed in collaboration with Saw Swee Hock School of Public Health and Health Promotion Board. **Appendix 2 HELIOS FFQ validation study** defines the FFQ validation study protocol.

3.4.7 *Reproducibility exercises*

As with any clinical procedure and scientific research, and as part of routine quality control, it is important to repeat health measurements in the same individuals after certain time to investigate the validity of our health measurements and ascertain quality of the HELIOS Study. Therefore, we plan to invite up to 600 past HELIOS participants with equivalent proportion of gender and ethnicity, and whose last HELIOS Study visit is within the range of 6 months to 2 years, to repeat the HELIOS Study assessment. Participant will be offered the same amount of token of appreciation as in the HELIOS Study, and a research health report.

3.4.8 *Sub-studies*

The HELIOS Study provides a great platform for various types of sub-studies which aim to build specific databases or biobanks using highly specialised and sophisticated technologies. These sub-studies will build upon the standard assessments and samples collected from the HELIOS Study main protocol and enrich the overall HELIOS Study database. Due to the complexity of these additional assessments, these sub-studies may not necessarily be done on the same day, and is optional. Participants who agree to participate in the sub-studies will need to sign a confirmatory informed consent. The following appendices describe the details of the collaborative projects open as sub-studies:

1. **Appendix 3. The Asian Skin Microbiome Program**
2. **Appendix 4. The MRI Brain and Body Imaging Program**
3. **Appendix 5. Developing and Validating Macronutrient Taste Preference Task**

3.4.9 *IT System*

Imperial College London (ICL) has developed an IT system to support the operation of a prospective cohort study. The decision was taken to develop a comprehensive middleware solution as a suitable product was not, and is not available on the open market. There are number of products that partially address the requirement, but not one that transcends the screening process. The development has taken over 4 years and a very significant investment; however, the result is a proven, comprehensive study IT system that could quickly be re-configured to meet the requirements of the study.

ICL, in consultation with leading experts, has carefully selected and integrated into the research centre IT system the automated capture of data from a range of clinical measurement devices. These were chosen based on: the quality and value of data they capture, comparability with other large studies and ease and reliability of use. The ICL research centre IT system manages: the consent process, clinical data collection (acquisition by direct interface, where possible), and biological sample collection.

The system has been successfully used in the Qatar Biobank study to deliver an 18 month pilot study, in which more than 2500 participants were recruited and screened. The clinical assessment incorporated both basic (e.g. blood pressure) and very sophisticated equipment (e.g. DXA, optical imaging, physical fitness test). The equipment has been directly interfaced to the ICL software, to prevent manual data input errors, where possible, and to allow real time analysis through the research process.

Importantly, additional commercially procured components can be linked to the ICL IT system, such as a high throughput LIMS system, and questionnaire and booking system software. ICL is offering to further develop the IT system to suit the needs of the HELIOS study at an agreeable cost to LKC Medicine. Preliminary discussion and evaluation of the system has taken place. Discussions to take this further are in progress.

3.4.10 *Summary and Conclusions*

The proposed HELIOS study will collect an extensive range of phenotypic information from 10,000 participants during the baseline recruitment visit, including health and lifestyle questionnaires, and extensive clinical and physical measurements, such as: anthropometry, blood pressure, lung function (spirometry), ECG, arterial stiffness, 3-D carotid ultrasound, ophthalmology assessment, physical fitness test, physical activity monitoring (accelerometer), hand grip strength, cognitive function, and DXA scan. Additionally, biological samples (blood, urine, saliva, skin tape and stool) will be collected from each participant. These rich baseline data, together with long-term participant follow-up, will enable the investigation of a wide range of potential disease risk factors in relation to well-being and ill-health in Singapore. Importantly, the HELIOS study will provide a world class resource for current and future generations of biomedical researchers from a wide range of disciplines.

3.5 **Project Phases**

3.5.1 ***Set-up and Testing Phase,*** until February 2018

a. Recruitment and training of staff
b. Sourcing and fit out of premises
c. Definition, procurement, and setup of equipment
d. Establishing the IT infrastructure, including hardware and software
e. Establishing the governance boards and advisory committees
f. Establishing the participant recruitment and booking system, contact centre, and promotion of the study

3.5.2 ***Recruitment Phase,*** from date of NTU IRB approval for 24 months

g. Commence recruitment:
    i. Registration of interest from prospective participants for the pre-pilot and pilot phase (up to 500 participants). We have provided an appendix containing a protocol for the pre-pilot phase (**Appendix 1**) and FFQ validation (**Appendix 2**).
    ii. Registration of interest for the main study (up to 10000 participants).
    iii. Recruiting volunteers for the pilot and the main study

h. Evaluate operational processes
i. Establish data quality monitoring processes
j. Establish mechanisms for data annotation and review
k. Collect participant feedback
l. Engage the local biomedical community in the study
m. Invite subject matter experts to review the collected data (and collection processes)
n. Identify areas for change/improvement; agree and plan the implementation of the change
o. Establish the capacity to consistently recruit and screen the targeted number of participants (based on a 240 day working year)
p. Establish the laboratory infrastructure to cope with the daily demand and long-term sample storage requirement (IT systems (LIMS), staff, automated sample processing equipment, automated cryogenic sample storage and retrieval equipment)
q. Monitor study performance (data quality and all study processes) continually and by undertaking study review after the first 3,500 participants have been recruited
r. Develop and implement infrastructure and processes to make data available to *bona fide* researchers

3.5.3 ***Statistical and epidemiological analyses of the data collected during recruitments***
from May 2018 onwards

3.6 Protection of Human Subjects

3.6.1 *Human Subject Involvement and Research Material*

The cohort will recruit adults 30 years of age or older. Participants will be invited to attend clinical assessments, during which the following measurements will be collected: self-reported health, lifestyle, and dietary behaviours via questionnaire; blood pressure; anthropometry; DXA; spirometry; electrocardiogram; treadmill fitness test; handgrip strength; cognitive function; arterial stiffness; and carotid ultrasound. Participants will also be issued accelerometers to collect activity data over a fixed period of time. In addition, blood, urine, saliva, and stool samples will be collected.

3.6.2 *Recruitment and Consent Procedures*

Participants will be provided with details of the measurements and data to be collected during the baseline visit, as well as being informed of the option to withdraw from the study at any time. Participants will be required to provide signatures on written, informed consent documentation before joining the study.

3.6.3 *Potential Risk/Benefits*

Standardized clinical procedures will be followed to minimize risk of injury and infection during biospecimen collection and clinical measurements. All data will be kept strictly confidential. No individual identifiers will be available to researchers requesting data for data analysis.

3.6.4 *Potential Benefits of the Proposed Research to the Participants and Others*

Participants will receive a feedback report on their clinical measurements which may result in beneficial health outcomes, such as referrals for further assessment and possible treatment as needed. For the epidemiological analysis of the cohort, there are few direct benefits except for the awareness of being involved in a large study that has the potential to improve understanding of risk factors associated with health outcomes in Singapore, ultimately leading to new treatments and prevention.

3.6.5 *Women and Minority inclusion*

The study will aim to recruit equal proportions of men and women. The study will oversample Malay and Indian participants in order to ensure sufficient sample size for subgroup analyses (recruitment plan: 60% Chinese, 20% Indian, and 20% Malay).

3.6.6 *Inclusion of children*

The focus of the cohort is the investigation of risk factors (diet, anthropometry, physical activity, lifestyle characteristics etc) during adult age and subsequent risk of common chronic diseases in Singapore, such as diabetes and heart disease, which are more prevalent among older populations. Therefore the study will not include any children.

3.7 Data management and data security

The personal details of participants will be stored separately from the research database, such that the database is in a linked de-identified format. The codes that match the personal and research identifiers will be held separately from both the personal and research data, in a secure location accessible only to the joint-Chief Investigators at NHG and NTU LKC Medicine, the designated Database Manager and their deputy. We will comply fully to PDPA requirements.

Use of the research data and samples will be regulated by the HELIOS Study "Scientific and Data Access Committee". This Committee comprises the named HELIOS Study PIs. Permission to Access to use the data will be evaluated based on written applications, according to scientific merit. Applications must be for specific research purposes, within the remit of the HELIOS research program approved by the IRB, and will provide time-limited access to the data needed to complete the research.

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Appendix 1 HELIOS study: pre-pilot and pilot phases version 2.0 dated 26/9/2017

HELIOS STUDY: PRE-PILOT AND PILOT PHASES

1. Aims of the pre-pilot and pilot phases

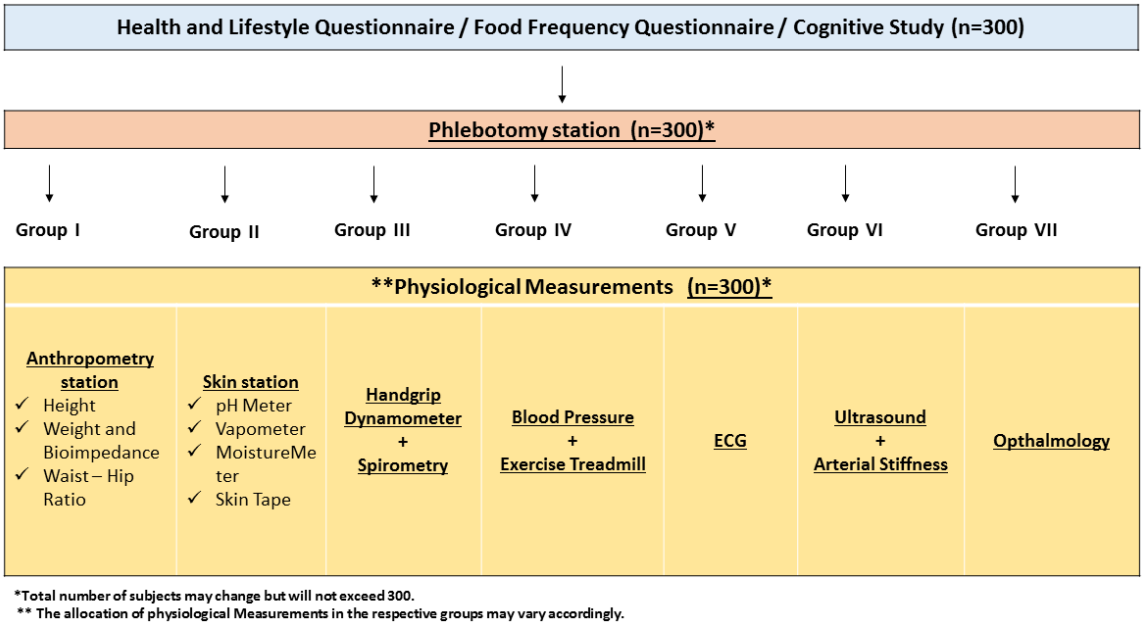
Table 1. The aims of the HELIOS pre-pilot and pilot phases

Pre-pilot	• Evaluate the quality of translation for questionnaire-related items. • Evaluate the acceptability of the individual assessment • Assess the duration of individual assessment • Assess the individual station workflow • Test the recruitment strategies • Investigate the data quality of physiological assessments and biological sample processing: reproducibility, intra- and inter-variability
Pilot	• Appraise the overall study workflow, total study duration and the acceptability of the full study protocol • Re-test and evaluate recruitment strategies

2. Summary of the procedures in the pre-pilot and pilot phases

Unless otherwise stated, the participant recruitment, inclusion and exclusion criteria will follow that of the main study protocol. During the **pre-pilot phase**, approximately 300 participants will be sub-grouped and assigned to some, but not all, of the stations in the HELIOS study (as shown in **Figure 1**). Overall, each participant would experience a mixture of the questionnaire-related items, physiological assessments and biological sample collection. Participants may take part in the pre-pilot studies more than once as long as they are completing different components of the pre-pilot evaluation. The number of times that participants could participate again is capped at 3. To ensure that participants have sufficient rest in between each session, we would advise participants to come on a separate day. In the **pilot phase**, 180 people will undergo a full study protocol.

Figure 5. **The Workflow of Pre-Pilot Phase,** for illustration purpose only.



*Total number of subjects may change but will not exceed 300.
** The allocation of physiological Measurements in the respective groups may vary accordingly.

During the pre-pilot phase, we will be assessing variability and reproducibility for each of the physiological measurements. To achieve this, participants will undergo repeated measurement of individual component of the study protocol (as summarised in **Table 2**). Participants will be given sufficient time to rest before undergoing the next repeated measurement.

Table 2. The list of repeated measurements per HELIOS station

Physiological assessments (stations)	Numbers of measurement per subject in the pre-pilot phase	Number of measurements per subject in the pilot phase
Blood pressure	12	3
Hand grip strength	24	6
Spirometry	16	4
Treadmill	1	1
ECG	4	1
Arterial stiffness	4	1
Height	10	1
Weight - Bioimpedance	10	1
Waist – Hip circumference	30	3
pH Meter	30	3
Vapometer	30	3
MoistureMeter	30	3

The investigation of sample quality, variability and reproducibility in sample processing will be done after the collection of samples, hence will not be detailed here. The types of biological samples that will be collected will be similar to the main study protocol. The blood volume or unit/ participant that will be collected will not exceed/ be less than those specified in the main study protocol (49.5 ml). **Table 3** describes the total number of participants from whom blood samples will be collected.

Table 3. Total number of participants for blood collection and breakdown of volume

Study Phase	Pre-pilot Phase	Pilot Phase
Total participants from whom samples will be withdrawn	n= 300 subjects (≤ 49.5 ml)	n= 180 subjects (49.5 ml)

3. Summary of Participant Benefits

Participants in the pre-pilot and pilot phases will receive SGD$50.00 as an inconvenience fee.

Since the aim of the pre-pilot phase is to train staff members, assess their competency, and validate the accuracy of the measurements across stations, we will not provide a screening report during the pre-pilot phase.

However if the supervising Principal Investigators (who are registered clinicians) identify clinically relevant findings that require urgent clinical follow-up, care will be taken to inform the individual immediately and he/she will be referred to specialised care or general practitioners accordingly.

Appendix 2 HELIOS FFQ validation study version 2.0 dated 26/09/2017

Validation of the HELIOS Study Food Frequency Questionnaire

1. Aims of the validation exercise

The aim of this validation study is to assess the accuracy of a new approach for measuring dietary habit in Singaporeans, using computer-based tools. We are evaluating two electronic tools: 1) a computer-based Food Frequency Questionnaire (FFQ) developed based on the latest paper-and-pencil FFQ in Singapore (Neelakantan et al., 2016) and 2) the mobile phone-based "Snap and Eat" App developed recently by the Health Promotion Board (HPB) which asks you to record what you eat, including through photographs.

1.1. FFQ validation study participants

We will recruit ~220 people to the FFQ validation study in 2 groups, comprising an initial 40 participants for the evaluation of translation quality, and a further 180 participants for a formal validation exercise. Participants will be recruited from amongst people attending the HELIOS study or other interested parties meeting HELIOS study entry criteria.

Group	Sample size	Experimental aim
1	n=40	To evaluate the quality of translation of FFQ using a cognitive interview
2	n=180	To administer FFQ validation study sessions

Group 1: Participants will be interviewed by the HELIOS study RAs using a qualitative approach to assess the understanding of the translated texts, the acceptability and the usability of the digital application, and evaluate whether any aspect of the system needs to be redesigned. This will take approximately 90 minutes per participant.

Group 2: Participants will complete 7 FFQ validation study sessions over the course of 7 months (2 of these sessions are done at home, 5 sessions are done in the HELIOS screening center). The FFQ validation study sessions are as follows:

Session	Study month	Location	Time (min)	Program
1	1	Screening centre	60	interview-based or electronic Food Frequency Questionnaire (FFQ), randomly assigned, collection of fasting blood
2	2	Screening centre	60	Face-to-face diet interview (24 hour dietary recall)
3	3	Home	varies	Use HPB Snap and Eat App for 3 days
4	4	Screening centre	60	Face-to-face diet interview (24 hour dietary recall)
5	5	Home	varies	Use HPB Snap and Eat App for 3 days
6	6	Screening centre	45	Face-to-face diet interview (24 hour dietary recall)
7	7	Screening centre	60	interview-based or electronic FFQ, randomly assigned, collection of fasting blood

We will administer 2 types of FFQ, the interview-based and the electronic version, using a cross-over design in Sessions 1 and 7. This will allow us to both 1) compare different format of FFQ administration and 2) evaluate the reproducibility of the FFQ.

Number of participants	Session 1	Session 7
45	Electronic FFQ	Interview FFQ
45	Interview FFQ	Electronic FFQ
45	Electronic FFQ	Electronic FFQ
45	Interview FFQ	Interview FFQ

At sessions 2, 4 and 6 we will interview participants in detail about what they ate/drank in previous 24 hours (a 24-hour dietary recall). Two of the 24-hour recall sessions will be carried over the weekdays and one will be over the weekend (or non-working days). Three sessions of 24-hour dietary recall are necessary as previous studies show this improves the accuracy of dietary assessments (Neelakantan et al., 2016).

Fasting blood (17.5 ml) will be collected at sessions 1 and 7 to facilitate the measurement of dietary biomarkers (e.g. fatty acids, amino acids, vitamins) and comparison to estimates of the dietary intake from the FFQ. The samples will be stored in -80^{o} prior to analysis in a pseudo-anonymised format (alpha-numeric label, no personal identifiers).

The Snap and Eat app is a freely downloadable App, and participants will be asked to record their dietary intake by taking a photo of what they eat, categorise them according to the mealtime and type, and upload them to the App. Data stored are initially collected by the HPB database, and will be transferred to HELIOS team for analysis. The Snap and Eat App has been launched as part of the HPB's national campaign to promote health diet among Singaporeans, but this will be the first time that the App is scientifically validated.

2. Summary of Participant Benefit and Inconvenience Fee

Upon completion of the study sessions, participants will receive $100.00 and a summary of their nutritional intake along with a written guidance on healthy eating according to HPB recommendation.

Reference

Neelakantan N, Whitton C, Seah S, Koh H, Rebella SA, Lim JY, Chen Sq, et al. 2016. Development of a Semi-Quantitative Food Frequency Questionnaire to Assess the Dietary Intake of a Multi-Ethnic Urban Asian Population. *Nutrients*. 8:528.

Appendix 3 The Asian Skin Microbiome Program

The Asian Skin Microbiome Program is a collaborative study involving LKC Medicine, A*STAR, and Skin Research Institute of Singapore (SRIS), which aims to better understand the factors that determine the health and wellbeing of skin in Asians. The protocol for this sub-study includes: i) skin physiological assessments; ii) skin examination; iii) skin surface sampling (by skin tapes) to collect the DNA of microbial flora, lipids, and microbes for culture, and/or metabolites; iv) skincare questionnaire; and v) skin imaging. Participation in this sub-study is optional, and participants who agree will be asked to sign a confirmatory consent form, and assessed on the same HELIOS Study visit for approximately 20-30 minutes.

Skin physiological assessments consist of 1) trans-epidermal water loss (TEWL) by vapometer, 2) surface hydration by MoistureMeter SC, and 3) skin surface pH, all of which are derived from the HELIOS Study. Additionally we will measure skin sebum secretion level by sebumeter. Each parameter comprises three readings each from inner forearm (derived from HELIOS Study) and cheek. The skin examination involves visual screening, which will also be derived from the HELIOS Study.

The skin surface sampling will be performed using industry standard tapes and swabs. In addition to the collection of skin tapes in the HELIOS Study from forearm, antecubital fossa and back, the sub-study will collect samples on the following sites: leg, face, scalp, axilla (under arm), inguinal crease. The procedure is not expected to create any pain.

The skincare questionnaire consists of questions that ascertain the presence of any cosmetic or clinical skin conditions, their perceived severity, the subject's perception of their overall skin health, and specific clinical diagnostic criteria. Female participants will be asked to answer additional detailed questionnaire regarding their menstrual cycle.

Skin imaging will be done using the VISIA® system to measure spots, wrinkles, texture, pores, UV spots, brown spots, and other measures of skin health on the face.

The data and samples obtained will be stored in line with the approved HELIOS Study guideline.

Depending on the results of the skin samples, participant may be contacted for a follow-up assessment on another day. This will include additional collection of skin tapes/swab from arm, back, leg, face, scalp and underarm as well as follow-up measurements of skin water level, skin observation and skincare questionnaire. This follow-up assessment is optional. The additional assessment will take about an hour.

Appendix 4 The MRI Brain and Body Imaging Program

The HELIOS Brain and Body Imaging Program aims to collect the MRI images of the brain and body. This information will be used to better understand the trajectories for brain health, maturation and ageing and can be used to better predict or prevent various diseases including Alzheimer's disease and diseases that affect other organs.

Participation in this sub-study is optional, and participants who agree will be asked to sign a confirmatory consent form. Participants who consent will be asked to attend a MRI scan on a separate day in the Center for Cognitive Neuroimaging centre (**CoNiC**), Level 7, Experimental Medicine Building, NTU LKC Medicine Yunnan Garden campus. CoNiC is part of the Centre for Neuroimaging Research (**CeNReN**) that is headed for Prof. Balasz Gulyas, Professor of Translational Neurosciences in NTU LKC Medicine. The centre is experienced in conducting human MRI scan for neurological research purpose. The session is one-time visit only, and is expected to last about 2 hours.

The MRI scan will be done using Siemens 3T PRISMA (or equivalent). Briefly, participants' body will be imaged from brain to pelvis area. The scan protocol that is used will be able to visualise small lesions, inflammation and cysts but will exclude cardiac and functional imaging (fMRI).

The data obtained will be stored in line with the approved HELIOS Study guideline.

The contraindications to MRI scan will include: presence of pacemaker/ defibrillation devices and cochlear implants, insulin pumps, and history of working with sheet metal.

We will also explain to participants that there are risk associated with claustrophobia (fear of small space leading to psychological distress) and risk associated with tattoos (transient skin irritation, possible cutaneous swelling or heating sensation at the site of tattoos. Participants will undergo a familiarization session under a Mock MRI machine to screen for any signs of discomfort or distress prior to proceeding on with the study.

The assessments are being done purely for research purposes. The results of these research tests may not always be suitable for clinical decision making, and as a result, we will not routinely report these back to participants. However in line with the incidental finding policy, if the research team do identify an abnormality during the course of participants' HELIOS assessment, which they believe might have important clinical significance, we will feed back the results.

Appendix 5 Developing and Validating Macronutrient Taste Preference Task

1. Background and rationale

Obesity, or excess fat deposition, is a known risk factor for hypertension, hyperlipidaemia, and type 2 diabetes, and is generally a consequence of the imbalance between energy intake and expenditure. Adults with obesity had 55% higher risk of developing depressive disorders [1]. One of the depressive symptoms is poor appetite or over-eating [2], and some studies performed in <500 individuals suggested that higher levels of depressive symptoms are associated with less ability to control eating [3,4], but what drives such extremely diverse outcomes (under- vs. over-eating) is not well understood. Appetite and taste preference are increasingly shown to be genetically driven [5,6]. However, to what extent these genetic predispositions translate to actual dietary intake and eventually obesity remains to be further investigated. Here we want to develop and validate a computerized macronutrient and taste preference ranking task (MTPRT) in Singapore, as part of a broader research aspiration to collect macronutrient and taste preference information in HELIOS Study

2. Methodology

MTPRT is a computerized test presenting 32 standardized food images of 4 macronutrient categories: high-carbohydrate, high-fat, high-protein, and low-energy [7]. Research participants will be required for 2 stages: 1) the development and 2) the validation of MTPRT. We will focus on the MTPRT development. The shortlisting of food items will be determined using the local nutrient composition compendium hosted by Health Promotion Board, as well as a list of commonly consumed food items is also available from the locally validated Food Frequency Questionnaire (FFQ) [8] used in the HELIOS study. Food photo-taking may be conducted if current picture stocks are deemed not suitable. We would then draft the computer test using questionnaire software. Subsequently we would conduct cognitive interview. The expected duration of the interview is 30 minutes., on one-on-one basis. The interview will evaluate these aspects: 1) suitability of food item choices, 2) suitability of food portions, 3) clarity of food pictures, 4) cultural representation, 5) formatting of the image presentation, 6) clarity of accompanying instruction, and 7) user-friendliness. We plan to interview 20 research participants with 1:1:1 Chinese: Malay: South Asian ethnicity ratio, 1:1 male: female ratio, and 1:1:1 30-39:40-49:>50 years age ratio. Participants will be recruited from samples of convenience (friends and relative) and/or from the HELIOS Study (offered on the same visit day at the end of HELIOS Study).

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