

# Effects of a single bout of walking on postprandial triglycerides in men of Chinese, European and Japanese descent: a multisite randomised crossover trial

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## ABSTRACT

**Introduction** Elevated non-fasting triglyceride (TG) concentrations are a risk factor for cardiovascular diseases but can be reduced after acute exercise. Ethnic-based differences in the magnitude of postprandial lipaemia and the extent that acute exercise reduces postprandial TG are poorly characterised across some ethnicities including those of East Asian origin. This paper describes the protocol of a multisite randomised crossover study comparing the effect of acute walking on postprandial TG in two groups of East Asian men with European men.

**Methods and analysis** Twenty Japanese, 20 Singaporean Chinese and 20 white British healthy men (21–39 years) recruited from Japan, Singapore and the UK, respectively, will complete two, 2-day trials. Fasted and postprandial venous blood samples and arterial blood pressure measurements will be taken over 6 hours the day after either: (1) 60-min treadmill walking; or (2) a rest day of normal living. The primary outcome is the difference in postprandial TG among ethnic groups after rest and walking. Secondary outcomes include cholesterol, glucose, insulin, ketone bodies, preheparin lipoprotein lipase, C-reactive protein and systolic/diastolic blood pressure.

**Ethics and dissemination** The study was approved by the Ethics Review Committee on Research with Human Subjects of Waseda University and the Nanyang Technological University Institutional Review Board. Relevant approval will be obtained from the UK site. Research findings will be disseminated through peer-reviewed journal publication and health conferences.

**Trial registration number** UMIN000038625.

## INTRODUCTION

Triglyceride (TG) is the predominant form of lipid in the circulation and concentrations are highest in the postprandial period (after eating). There is strong evidence from prospective cohort studies that postprandial TG concentrations are a risk factor for cardiovascular diseases,<sup>1 2</sup> and disturbances in postprandial lipid metabolism contribute

to the progression of atherosclerosis over the lifespan.<sup>3</sup> Systematic reviews and meta-analyses show that exercise is an effective strategy for reducing postprandial TG.<sup>4–6</sup> Much of the reduction is from the acute effect of exercise with rapid increases in postprandial TG apparent in the absence of any recent exercise.<sup>4–6</sup>

The prevalence of cardiovascular disease varies considerably across different countries and ethnicities.<sup>7</sup> Direct ethnic comparisons of postprandial metabolic responses to acute exercise are sparse, but two notable exceptions from the UK compared responses in individuals of South Asian and white European descent.<sup>8 9</sup> This work highlighted that South Asian individuals—a population with an increased susceptibility to coronary heart disease (CHD) and type 2 diabetes<sup>10 11</sup>—exhibited markedly higher postprandial TG and insulin than their white European counterparts. Furthermore, a single bout of running reduced postprandial TG to a greater extent in the South Asian compared with the white European men,<sup>9</sup> whereas acute brisk walking induced an equivalent reduction in postprandial TG and insulin between the ethnicities.<sup>8</sup> Evidence has also demonstrated that South Asian men have higher skeletal muscle expression of genes involved in oxidative metabolism and a greater capacity for mitochondrial ATP production.<sup>12</sup> However, at the same time whole-body fat oxidation during submaximal exercise appears lower than in Europeans.<sup>13</sup> Whether such differences impact exercise-induced reductions in postprandial TG between ethnicities is uncertain.

Examining differences in CHD risk factors by ethnicity is important as current exercise guidelines do not distinguish recommendations based on ethnicity.<sup>14–18</sup> This should

include investigations in more diverse and understudied ethnic groups such as those of East Asian origin. This is because previous studies have shown that the absolute value of some risk markers (eg, insulin sensitivity, body fat percentage, body mass index (BMI) cut-offs for detecting metabolic abnormalities) for CHD differ between East Asian and white European groups,<sup>19 20</sup> and even between South Asian and East Asian groups.<sup>21</sup> In addition, physical activity guidelines in East Asian countries are based on data derived mainly from Western countries<sup>16 17</sup> even though the response to physical activity may not be the same in East Asians. The importance of diversifying ethnic-based research to East Asian populations is underscored by the upward trajectory in adverse CHD risk factors including dyslipidaemia, diabetes and obesity apparent in these populations in recent years.<sup>22 23</sup> The rationale is strengthened further by evidence demonstrating that levels of physical inactivity are high in high-income East Asian countries including Singapore and Japan as well as in European countries.<sup>24</sup>

Low-intensity to moderate-intensity walking is frequently advocated as a strategy to augment physical activity engagement and to promote metabolic health benefits at the population level.<sup>14–18 25</sup> Furthermore, walking, particularly briskly, is associated with a lower risk of cardiovascular disease,<sup>26</sup> and favourable reductions in postprandial TG have been reported after acute bouts of walking in studies involving individuals exclusively of Singaporean,<sup>27</sup> Japanese<sup>28–30</sup> or European<sup>31 32</sup> ancestry. However, further work is required to directly compare the acute effects of walking on postprandial CHD risk factors in individuals of East Asian and European descent. This study aims to expand the current evidence base by adopting an international multisite approach. This will allow direct comparison of fasting and postprandial cardiometabolic risk markers in response to acute exercise between men of Singaporean Chinese, Japanese and white British descent.

## AIMS AND OBJECTIVES

### Aim

The aim of this multisite randomised crossover study is to compare the acute effects of walking on fasting and postprandial markers of CHD risk in men originating from three distinct ethnic groups: (1) Singaporeans of Chinese descent; (2) Japanese and (3) white British.

### Primary objective

To investigate the impact of a single 60-minute walking bout on postprandial TG concentrations in Singaporean Chinese, Japanese and white British men.

### Secondary objectives

To investigate the impact of a single 60-min walking bout in Singaporean Chinese, Japanese and white British men on fasting and postprandial:

1. Blood lipids (total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, non-esterified fatty acids (NEFA)).

2. Ketone bodies (3-hydroxybutyrate (3-OHB), acetoacetic acid, total ketone body).
3. Preheparin lipoprotein lipase (LPL).
4. Glucose and insulin.
5. C-reactive protein (CRP).
6. Resting brachial arterial blood pressure.
7. Ratings of perceived appetite.

The secondary aim of this study is to investigate ethnic-based differences between Singaporean Chinese, Japanese and white British men in:

1. Body adiposity (sum of five skinfold thicknesses, waist circumference, BMI).
2. Treadmill-based maximum oxygen uptake.
3. Whole-body substrate oxidation during submaximal exercise.
4. Habitual levels of physical activity using accelerometry.

## METHODS AND ANALYSIS

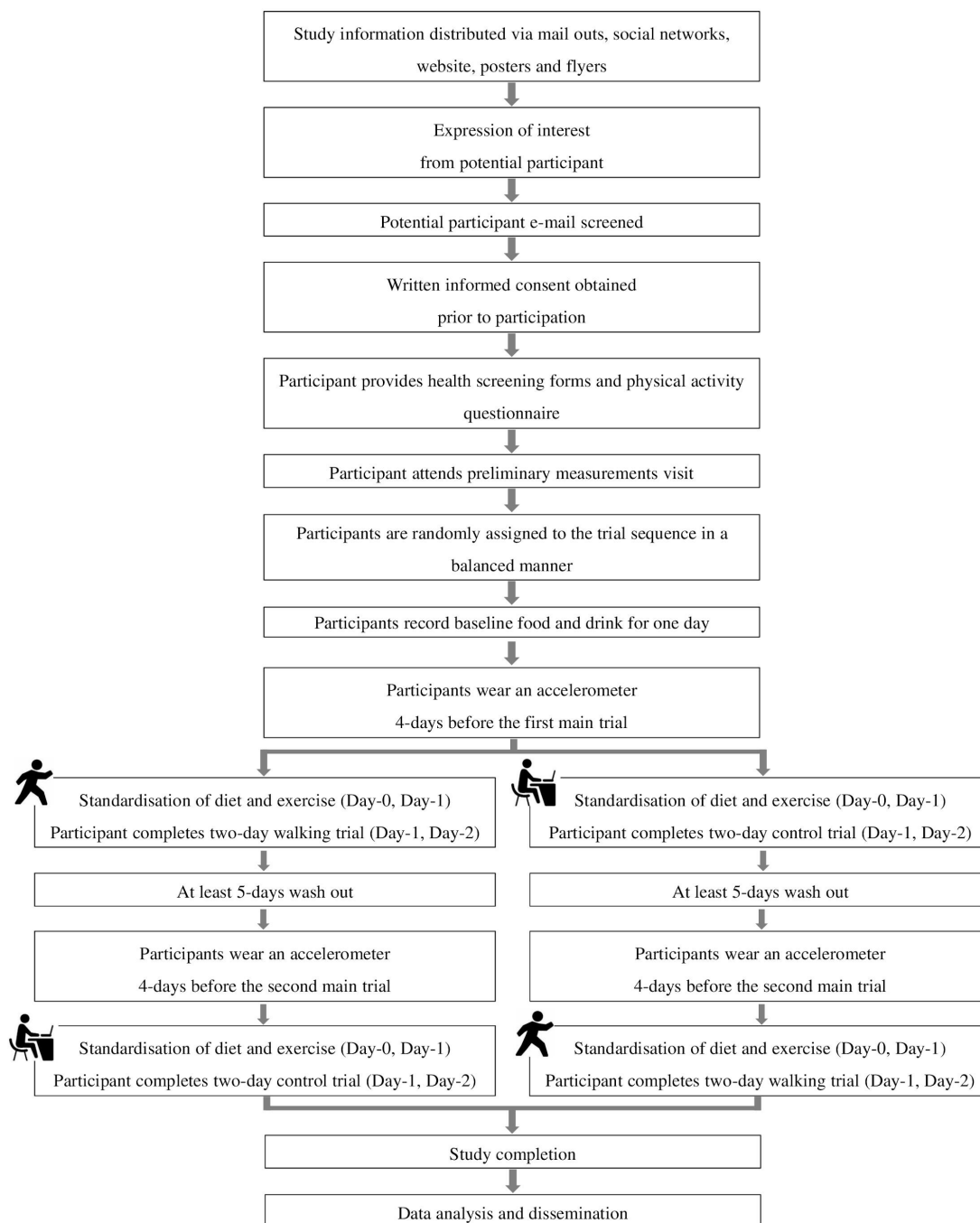
### Study design and setting

This will be a multisite laboratory-based randomised crossover study conducted in accordance with the Standard Protocol Items: Recommendations for Interventional Trials statement.<sup>33</sup> The study schedule is shown in [figure 1](#). Research will take place at three institutions: Waseda University (Japan), Nanyang Technological University (Singapore) and Loughborough University (UK). Ethical permission for the study will be obtained at each site of research. Eligible participants who have completed informed consent, screening and preliminary testing will be invited to complete two, 2-day trials in a random order. The lead investigator (CN) will enrol participants to the study and randomly assign the trial sequence in a counterbalanced manner at each study site using computer-generated random numbers to avoid order effects. Due to the nature of the study design, it will not be possible to blind researchers or participants to the trial order allocation. Each trial will be separated by  $\geq 5$  days to eliminate any potential carry-over effects.

### Participants

#### Inclusion criteria

Individuals who are male, aged 21–39 years, either Japanese (Waseda University), Singaporean Chinese (Nanyang Technological University) or white British (Loughborough University), non-smokers, sedentary (leisure-time physical activity:  $<150$  min per week of moderate-intensity physical activity or  $<75$  min per week of vigorous-intensity physical activity), and have a BMI of  $18.5$ – $27.5$  kg/m<sup>2</sup> will be eligible (ie, those who meet all of the criteria) to take part in this study. As CHD mortality in males is typically 4–5 times higher than females<sup>34</sup> and males generally develop cardiovascular diseases at a younger age than females,<sup>35</sup> we will only recruit males to this study. To verify their ethnicity, each participant will be asked verbally to provide details of their place and country of birth, mother tongue, race and the ethnicity of their parents and grandparents.



**Figure 1** Study recruitment and design.

### Exclusion criteria

Individuals will not be eligible for participation if they have any form of cardiometabolic disease (eg, cardiovascular disease, diabetes, metabolic syndrome, hypertension), show any symptoms of (eg, chest pain) or have any medically diagnosed contraindications to exercise testing, have any balance or dizziness problems, have any chronic medical conditions (whether medicated or not), have any bone or joint problems, are taking any medication(s) known to influence the study outcomes or have any allergies to food items used in the test meal.

### Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting or dissemination plans of this research.

### Recruitment

Volunteers will be recruited through mail outs, social networks, websites, posters and flyers. Volunteers expressing interest via email will be asked basic screening questions (eg, height, body mass, physical activity level) to assess their initial suitability. Those deemed potentially eligible will be invited to complete written informed

consent and a full screening session at a laboratory within each institution. Written informed consent will be obtained by a member of the research team before participation in any testing protocols (Appendices 1 and 2). Each volunteer agreeing to enrol will complete a standard health screening form to gather information on medical history and medication use, and the International Physical Activity Questionnaire (short form).<sup>36</sup>

### Preliminary measurements

#### Anthropometric measurements

Body mass will be measured to the nearest 0.1 kg using digital scales (Japan site: Inner Scan 50, Tanita Corporation, Tokyo, Japan; Singapore site: Seca 764, Seca GmbH & Co. KG., Hamburg, Germany; and UK site: Seca 285, Seca GmbH & Co. KG, Hamburg, Germany). Height will be measured to the nearest 0.1 cm using a wall-mounted stadiometer (Japan site: S-OA, AS ONE Corporation, Osaka, Japan; Singapore site: Seca 764, Seca GmbH & Co. KG., Hamburg, Germany; and UK site: Seca 285, Seca GmbH & Co. KG, Hamburg, Germany). Waist circumference will be measured to the nearest 0.1 cm at the level of the umbilicus using a flexible plastic tape. Skinfold thickness will be measured at five sites (subscapular, triceps, chest, abdomen and thigh) on the right side of the body with the use of a calliper (all sites: MK-60; YAGAMI, Nagoya, Japan), and values will be combined to derive the sum of five skinfold thicknesses. Resting arterial blood pressure will be measured using a mercurial sphygmomanometer and a digital monitor (further details provided in 'Analytical methods: Blood pressure' section).

#### Exercise tests

After collecting anthropometric measures, participants will undergo two exercise tests. The first test will involve a 16-min treadmill test to establish the relationship between walking/jogging speed and oxygen uptake. Participants will complete four, 4-min incremental stages starting at a speed of 4 km/h and increasing by 2 km/h every 4 min; the treadmill gradient will be set at 0% throughout. After resting for 20 min, participants will then complete a maximum oxygen uptake test. The speed of the treadmill will be constant during the test depending on the participants fitness level. The initial gradient will be 3.5% and increase 2.5% every 3 min until voluntary exhaustion. Oxygen uptake will be measured continuously throughout the test using an online breath-by-breath gas analyser (Japan site: Quark RMR, COSMED Co., Roma, Italy; Singapore site: True One 2400, Parvo Medics, Utah, USA; and UK site: METALYSER 3B, Cortex, Leipzig, Germany). Heart rate will be measured continuously throughout the tests using short range telemetry (all sites: Polar M400, Polar Electro Japan Co., Kempele, Finland). Ratings of perceived exertion will be recorded at the end of each stage during both exercise tests using a subjective 6–20 scale.<sup>37</sup>

#### Habitual physical activity

Participants' habitual physical activity level will be determined using a uniaxial accelerometer (all sites: Lifecoder GS; Suzuken Co., Nagoya, Japan).<sup>38 39</sup> This uniaxial accelerometer determines the number of steps and the physical activity level (1–9) per day.<sup>38</sup> Participants will wear the accelerometer continuously on the hip during the 4 days before day 0 of each main trial (total duration: 8 days). Based on a previous study,<sup>38</sup> an activity intensity of 1–3, 4–6 and 7–9 indicates light (<3 metabolic equivalents (METs)), moderate (3–6 METs) and vigorous (>6 METs) intensity physical activity, respectively. Analysis will be restricted to participants who provide at least 3 days (2 weekdays, 1 weekend day) of valid accelerometer data and at least 10 hours of valid daily wear time will be required for a valid day.

### Main trials

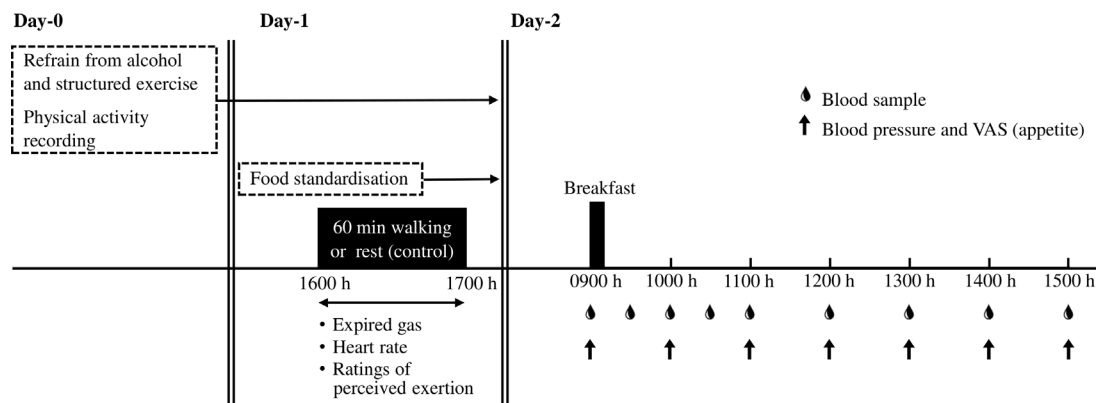
#### Standardisation of diet and exercise

Participants will weigh and record all food and drink consumed for 1 day at baseline in advance of their first main trial. Participants will be asked to replicate this diet on day 1 of both main trials for standardisation. Food diaries will be analysed by a registered dietitian to determine energy intake and macronutrient content. Participants will be instructed to refrain from alcohol and structured exercise on day 0 and day 1 (apart from the exercise performed as part of the experiment on day 1). Participants will wear the accelerometer continuously on the hip on day 0 and day 1 of each main trial to compare their physical activity between trials. Participants will receive text messages from a researcher to replicate their dietary intake on day 1 and physical activity patterns on day 0 and day 1 of each main trial with compliance checked verbally on arrival at the laboratory on day 2 of each trial.

#### Experimental protocol

A schematic illustration of the experimental protocol is shown in [figure 2](#). On day 1 of each trial, participants will come to the laboratory before 1600 hours and resting arterial blood pressure will be measured in a seated position with legs uncrossed and both feet flat on the floor. Participants will then complete one of the following interventions in a random order: (i) walk on a treadmill at a speed and gradient equivalent to 40% of their maximum oxygen uptake (determined from the preliminary test) for 1 hour (ie, from 1600 hours to 1700 hours) (walking); or (ii) rest for 1 hour in the laboratory (ie, from 1600 hours to 1700 hours) (control). Oxygen uptake, heart rate and perceived exertion will be measured during the walking trial as described previously. The exercise gross energy expenditure and substrate oxidation will be estimated using the equations of Frayn.<sup>40</sup> In the control trial, participants will engage in passive activities while resting in the laboratory (sit quietly reading, working or playing with hand-held electronic devices). During both trials, participants will be asked to replicate their prerecorded evening meal on day 1 after





**Figure 2** A schematic representation of the study protocol. On day 1 of the walking trial, participants will carry out their usual free-living activities throughout the day and complete 1 hour of brisk walking in the laboratory from 1600 hours to 1700 hours. The control trial will be identical except that participants will rest in the laboratory from 1600 hours to 1700 hours. On day 2 of both trials, participants will consume a standardised breakfast and then sit in a chair (reading, writing, working at a computer or watching television) in the laboratory until 1500 hours. VAS, visual analogue scales.

leaving the laboratory and then fast overnight for 10 hours (no other food or drinks except water).

On day 2 of each trial, participants will transport themselves to the laboratory by public transport or car (not walk or cycle). On entering the laboratory, measurements of body mass, resting arterial blood pressure and ratings of appetite and taste using visual analogue scales<sup>41</sup> will be collected. Participants will then sit on a chair or lie on a bed while a cannula is inserted into an antecubital or forearm vein. After 10 min of rest, a 10 mL blood sample will be collected. Participants will then consume a standardised breakfast. A clock will start with the first bite of the breakfast and participants will be allowed 20 min to consume the meal. Further 10 mL blood samples will be collected at 0.5, 1, 1.5, 2, 3, 4, 5 and 6 hours after the start of breakfast. In addition, resting arterial blood pressure and ratings of perceived appetite and taste will be collected at hourly intervals. At the end of the 6 hours, the cannula will be removed and participants will be free to leave the laboratory. The ambient temperature and relative humidity during the main trials will be recorded.

Participation in the study will discontinue and the data removed from the final analysis if any participant: (i) is unable to comply with our instructions/directions, (ii) is unable to complete the exercise tests, (iii) is unable to complete both trials or (iv) decides to withdraw from the study.

#### Test meal and water consumption

The test meal will be a continental breakfast consisting of a salad (lettuce, cucumber, tomato and ham with French dressing), white bread with butter, soup (corn soup powder, skim milk and hot water), scrambled egg (egg, whole milk, butter and salts with tomato ketchup), cornflakes with whole milk and orange. The breakfast will provide 0.38 g fat (saturated, monounsaturated and polyunsaturated fatty acid contributions to the test meal will be 0.15 g, 0.10 g and 0.04 g per kilogram of body mass, respectively), 1.20 g carbohydrate, 0.37 g protein and 41 kJ (10 kcal) energy per kilogram of body mass (ie, the energy and macronutrient content of the test meal will be adjusted based on the participant's body mass). The macronutrient percentage of the breakfast will be 35% fat, 50% carbohydrate and 15% protein (table 1). This

**Table 1** Energy and macronutrient content of the test breakfast

	Per kg of body mass	Macronutrient percentage	eg, actual amount for 70 kg of body mass
Energy	41 kJ (10 kcal)		2860 kJ (684 kcal)
Fat	0.38 g	35	26.8 g
Saturated fatty acids*	0.15 g		10.8 g
Monounsaturated fatty acids*	0.10 g		7.2 g
Polyunsaturated fatty acids*	0.04 g		2.9 g
Carbohydrate	1.20 g	50	84.3 g
Protein	0.37 g	15	25.8 g

\*These values represent the amount of saturated, monounsaturated and polyunsaturated fatty acids calculated by the nutrition software and, therefore, do not add up to the total fat content.

test meal menu was chosen based on the assumption that it could be eaten by all three ethnic groups and that items could be easily purchased locally or shipped to Singapore and the UK. The energy and macronutrient composition of the test meal are based on actual total energy intake and macronutrient composition in the three ethnic groups as reported from national nutrition surveys conducted in Singapore, the UK and Japan.<sup>22 42 43</sup> The investigators will use exactly the same food items, except fresh foods and dairy products, at all three research sites. Participants will be asked to consume the meal within 20 min in a pre-determined order (salad, white bread and soup, scrambled egg, cornflakes and orange), and consumption time and order will be recorded and replicated in the subsequent trial. Participants will be supplied water in increments during the first trial, and the pattern and volume ingested will be replicated in the subsequent trial.

## Analytical methods

### Blood samples

For serum TG, NEFA, total-cholesterol, LDL-cholesterol, HDL-cholesterol, 3-OHB, acetoacetic acids, total ketone body, high-sensitivity CRP and LPL measurements, venous blood samples will be collected into tubes containing clotting activators for isolation of serum. Thereafter, samples will be allowed to clot for 30–45 min at room temperature and then centrifuged at 3000×g for 10 min at 4°C. Serum will be removed, divided into aliquots, and stored at –80°C for later analysis. Venous blood samples will be collected into tubes containing dipotassium salt–EDTA for plasma insulin measurements and into tubes containing sodium fluoride–EDTA for plasma glucose measurements. Thereafter, both tubes will be immediately centrifuged and plasma divided into aliquots for storage as previously described.

Enzymatic, colorimetric assays will be used to measure serum TG (Pure Auto S TG-N; Sekisui Medical Co, Tokyo, Japan), serum NEFA (NEFA-HR; Wako Pure Chemical Industries, Osaka, Japan), serum 3-OHB (KAINOS 3-HB; Kainos Laboratories, Tokyo, Japan), serum acetoacetic acids (KAINOS 3-HB; Kainos Laboratories, Tokyo, Japan), serum total ketone body (KAINOS TKB-L; Kainos Laboratories), plasma glucose (GLU-HK(M); Shino-Test Corporation, Kanagawa, Japan), total cholesterol (Determiner L TCII, Hitachi Chemical Diagnostics Systems Co, Tokyo, Japan), LDL-cholesterol (MataboLead LDL-C, Hitachi Chemical Diagnostics Systems Co, Tokyo, Japan) and HDL-cholesterol (MataboLead HDL-C, Hitachi Chemical Diagnostics Systems Co, Tokyo, Japan). ELISA will be used to measure plasma insulin (Mercodia Human Human ELISA Kit; Mercodia AB, Uppsala, Sweden) and serum preheparin LPL (LPL ELISA; Cell Biolabs, San Diego, CA). A latex agglutination assay will be used to measure plasma CRP (N-Assay LA CRP with high-sensitivity detection, Nittobo Medical Co., Tokyo, Japan). Samples collected in Singapore and the UK will be shipped to Japan and all analyses will be conducted at

Waseda University. All analysis for each participant will be completed within the same run for each measure.

### Blood pressure

Brachial arterial blood pressure will be measured in a seated position on the same arm during each trial using a mercurial sphygmomanometer (Japan site: MRP Automatic Cock System 605 P, KENZMEDICO Co., Saitama, Japan; Singapore site: Mercurial Sphygmomanometer CK-101C, Spirit Medical Co., Taipei, Taiwan) and a digital blood pressure monitor (all sites: OMRON HEM-907, Omron Healthcare Co., Kyoto, Japan). The ethnicity comparison will be conducted using the data collected from the same digital blood pressure monitor at all sites because devices containing mercury are prohibited in Europe. The concurrent measurement using the mercurial sphygmomanometer in Singapore and Japan will permit the validation of the data collected using the digital monitor. Participants will be seated on a chair for 10 min before each measurement. The average of two measures will be taken at each time point and the mean value recorded for statistical analysis.

### Ratings of perceived appetite and taste

Subjective feelings of hunger, satiety, fullness, prospective food consumption and desire to eat fatty, salty, sweet or savoury items will be assessed using visual analogue scales.<sup>41</sup> All visual analogue scales are 100 mm long and the scales are anchored by words describing the extreme positive and negative feelings of the construct being assessed.

## Study outcomes

### Primary outcome

The primary outcome will be time-averaged total area under the curve (AUC) for TG which will be calculated from the TG concentration determined at every blood sampling time-point on day 2.

### Secondary outcomes

Several secondary outcomes will also be assessed on day 2 of the control and walking trials. Concentrations of insulin, glucose, NEFA, 3-OHB, acetoacetic acids, total ketone body, preheparin LPL and CRP will be measured in all samples and presented as fasted and postprandial values. Total cholesterol, LDL-cholesterol and HDL-cholesterol will be measured in fasting samples only. Resting arterial systolic and diastolic blood pressure, and ratings of perceived appetite and taste will be collected fasted and postprandially at hourly intervals.

Additional secondary outcomes will be measured to permit demographic comparisons between ethnic groups involving body adiposity (sum of five skinfold thicknesses, waist circumference, BMI, maximum oxygen uptake, whole-body substrate oxidation during exercise and objectively measured habitual physical activity levels).

### Sample size calculation

Sample size calculations were performed using G\*Power V.3.1.0.<sup>44</sup> Previous research reported the between subject

effect (ethnicity: South Asian vs white European) (effect size, Cohen's  $d=0.97$  to  $1.22$  for total AUC for TG) using 60-min walking or running versus sedentary.<sup>8,9</sup> The sample size estimation was powered to detect an effect size of  $0.97$  using an unpaired t-test for comparison between ethnic groups. For three groups with an alpha level set at  $0.0167$ , an estimated total sample size of  $45$  (ie, 15 per group) would provide 80% power to detect between group differences. Previous research reported the within subject effect (trial: walking vs sedentary) (effect size, Cohen's  $d=0.82$  for total AUC for TG) using 60-min brisk walking vs sedentary.<sup>45</sup> The sample size estimation was powered to detect an effect size of  $0.82$  using a paired t-test for comparison between trials. For two trials with an alpha level set at  $0.05$ , an estimated total sample size of  $11$  would provide 80% power to detect between trial differences. Based on this, it is estimated that a total of  $45$  (ie, 15 per group) participants will be required for this two-trial crossover design. To allow for potential withdrawals, a total of  $60$  (20 per group) participants will be recruited.

### Statistical analyses

Data will be analysed using Predictive Analytics Software version 26.0 for Windows (IBM SPSS Statistics V.26.0). Normality of distribution will be assessed using the Shapiro-Wilk test. If data are not normally distributed, the data will undergo natural log transformation prior to analyses. Physical characteristics and exercise responses will be compared among ethnic groups using one-factor repeated-measures analysis of variance (ANOVA). Time-averaged total AUC over the 6-hour postprandial period on day 2 will be calculated for TG, glucose, insulin, NEFA, 3-OHB, acetoacetic acids, total ketone body, LPL and CRP using the trapezoidal method. The TG incremental AUC will be calculated using the same method after correcting for fasting concentrations in each trial. These values will be compared using two-factor mixed-ANOVA (between group factor: ethnicity, within subject factor: trial). A two-factor mixed-ANOVA, will also be used to assess between trial and ethnic group differences for fasting plasma/serum concentrations (TG, glucose, insulin, NEFA, ketone bodies, LPL, CRP, total cholesterol, LDL-cholesterol and HDL-cholesterol). Postprandial plasma/serum concentrations over time (TG, glucose, insulin, NEFA, 3-OHB, acetoacetic acids, total ketone body, LPL and CRP) will be compared using a three-factor mixed ANOVA (between group factor: ethnicity, within subject factors: trial and time point), with post-hoc analysis for multiple comparisons using the Bonferroni method. Potential confounding factors will be adjusted as covariates in the analyses if any physical or physiological characteristics known to affect fasting and postprandial metabolic responses (eg, age, BMI, waist circumference, body fat percentage) differ among the ethnic groups. If there are any missing time point data, a single imputation method will be used for statistical analysis. Effect sizes (Cohen's  $d$ ) for important post-hoc comparisons will be calculated to describe the magnitude of difference

between trials and ethnic groups. Effect sizes of  $0.2$  are considered the minimum important difference in all outcome measures,  $0.5$  moderate and  $0.8$  large.<sup>46</sup> Mean differences and the respective 95% CIs will be presented. Statistical significance will be accepted at the 5% level.

### Ethics and dissemination

#### Research ethics approval

This study was approved on 19 November 2019 by the Ethics Review Committee on Research with Human Subjects of Waseda University (Approval No: 2019-252), on 6 February 2020 by Nanyang Technological University Institutional Review Board (Approval No: IRB-2019-12-022) and will be approved by Loughborough University's Ethics Advisory Committee before data collection commences at the UK site.

#### Trial registration

This study was registered in advance with the University Hospital Medical Information Network Center (UMIN), a system for registering clinical trials. Investigators will be required to renew and report the progress and any modifications of the UMIN registration twice a year.

#### Protocol amendments

Any protocol modifications will be communicated to the Ethics Review Committee on Research with Human Subjects of Waseda University, Nanyang Technological University Institutional Review Board and Loughborough University's Ethics Advisory Committee as appropriate. Investigators will be required to annually renew and report the progress and any modifications of the study (the number of participants recruited, any adverse events, etc.) to the ethical review board at each site. All protocol amendments will be recorded in the UMIN-CTR and detailed in a journal publication of the study findings. Any individuals who are currently enrolled in the study at the time of the protocol change will be consented again with the updated version of the protocol as applicable.

#### Confidentiality

All data about potential and enrolled participants will be deidentified with each participant assigned a unique study code. Personal information (eg, name, address, contact details) will be stored separately from the deidentified data collected during the study. Data will be stored in electronic format on password-protected computers or in hard copy format in secure storage cabinets at Waseda University, Nanyang Technological University and/or Loughborough University. Only members of the research team will have access to this information. All information will be stored for a period of 10 years after publication. Individuals will be referred to in anonymised fashion in any published data. The data and blood samples will only be used for the purpose of the current study.

#### Ancillary and post-trial care

Investigators who identify or are informed of any serious adverse event will report the event to the responsible





Principle Investigator at each site (MM at the Japan site, SFB at the Singapore site and DJS at the UK site). The Principle Investigators will report any adverse events to the ethical review board at each site and institution specific guidelines will be followed.

### Dissemination

The findings of this research will be disseminated to lay, academic, practice and policy-based audiences via presentation at conferences, publication in a peer-reviewed international journal, websites and newsletters. The summary data will be made available as supplementary material when the findings of the study are published in a peer-reviewed journal. Researchers will put the final data set (ie, full raw data) analysed for any publications into a public data repository at the Singapore site. The result of individual data will be disclosed to all participants.

## DISCUSSION

The widespread benefits of physical activity in enhancing health and lowering the risk of chronic disease have been established abundantly across populations globally. Lifestyle-related chronic disease prevalence, including CHD, varies markedly across countries and ethnicities.<sup>7</sup> Despite this, direct ethnic comparisons of the health benefits of physical activity are sparse, and evidence-based physical activity guidelines are not ethnicity specific. Indeed, the physical activity guidelines in Singapore and Japan were developed primarily based on data from Western countries.<sup>16 17</sup> Recent trends demonstrate a rise in the prevalence of risk factors for CHD in individuals of East Asian descent<sup>22 23</sup> and therefore lifestyle strategies targeting modifiable risk factors could be crucial to mitigate future CHD risk in these individuals.

Single bouts of brisk walking can improve postprandial metabolism and other risk markers for CHD in different populations<sup>21–26</sup> but responses have not been compared directly in East Asian and white European individuals. This paper describes the protocol of a multisite randomised crossover study comparing the effect of acute walking on postprandial TG and other cardiometabolic disease risk markers in men of Singaporean Chinese, Japanese and white British descent.

One major novel aspect of this study is that participants will complete the study while living in their home country. This differs from previous investigations<sup>8 9</sup> where direct ethnic comparisons of the postprandial metabolic responses to acute exercise were conducted in a single country (ie, the UK). The importance of our approach should be emphasised since studies have previously shown that migrants often take on the behaviours of their host country. The change in lifestyle behaviours and environmental exposures (eg, dietary habits, physical activity levels and seasons) that occurs with migration<sup>47 48</sup> can affect CHD risk.<sup>49</sup> Thus, the current study will bypass this migrant effect and seeks to examine ethnic differences in postprandial TG and other cardiometabolic disease risk

markers in response to exercise when individuals are in their native environment.

There are several limitations to the present study. First, the evening meal on day 1 will not be standardised among participants as it is difficult to design a meal that can be prepared easily by participants using identical food items habitually consumed across different countries. Second, the use of different metabolic carts for determining maximum oxygen uptake at the three study sites could introduce variability in the prescription of exercise intensity and in the estimation of exercise energy expenditure and substrate oxidation. Nevertheless, the metabolic carts will be calibrated by a trained researcher according to the manufacturer's instructions using certified equipment for gas and volume calibration before each use to minimise any variability. Finally, prescription of exercise intensity based solely on the relationship between oxygen uptake and speed may elicit variable intensities between individuals depending on the protocol used and whether individuals are exercising above or below the lactate threshold.<sup>50</sup> However, our exercise protocol is standardised among sites and at the target exercise intensity prescribed (40% of maximum oxygen uptake), the rapid change in oxygen uptake kinetics with each increment in effort means that the predicted and actual intensity prescribed are likely to show good agreement.<sup>50 51</sup>

The data collected in this study will provide valuable information (1) to determine the efficacy of walking to promote beneficial changes in postprandial metabolism in three distinct ethnic groups; and (2) to diversify evidence on ethnic-based differences in postprandial metabolism and other CHD risk markers in individuals of East Asian and European ancestry.

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