The "metabolically-obese normal-weight" phenotype in two Asian ethnic groups and its reversal by calorie restriction and exercise

SPECIFIC AIMS & HYPOTHESES

The prevalence of overweight and obesity in Singapore is approximately half of that in the United States, yet the incidence of type 2 diabetes is similar, and is expected to double in the near future. This indicates that metabolic dysfunction, particularly insulin resistance, is widely prevalent even among individuals who are considered normal-weight or lean by conventional measures, i.e. body mass index (BMI) and percent body fat. These individuals are often referred to as "metabolically-obese normal-weight" (MONW), and have increased risk for cardiometabolic disease despite their normal BMI and total body fat values. The prevalence of the MONW phenotype varies across populations and differs markedly among different ethnicities (e.g. greater in Indian than in Chinese). However, our understanding of the complex interactions between ethnicity, body composition, and metabolic dysfunction and its reversal remains rudimentary. Previous attempts to characterize the MONW phenotype are confounded by the small but significant differences in BMI or percent body fat between groups (even if all subjects were lean, within the "normal" range), with MONW subjects being always "fatter" than the corresponding control subjects. There are no published studies that prospectively recruited groups of metabolically healthy and unhealthy lean individuals matched on BMI *and* percent body fat. Furthermore, although weight loss improves body composition and many of the cardiometabolic abnormalities in most obese patients, little is known about the possible therapeutic effects of calorie restriction in MONW subjects. Finally, regular endurance exercise can improve metabolic function, but its effects in MONW subjects are not known.

Accordingly, a better understanding of the MONW phenotype and the evaluation of therapeutic approaches for its reversal will have important implications for public health. By facilitating earlier identification of these subjects, who are more likely to go undiagnosed and thus less likely to be treated before clinically overt cardiometabolic disease develops, results from this study will allow for earlier and effective intervention. To this end, this project aims to test the following hypotheses:

Hypothesis 1: We hypothesize that, compared with metabolically-healthy lean subjects, MONW subjects have reduced insulin-mediated glucose disposal, impaired β-cell function, increased intra-abdominal adipose tissue, more ectopic fat deposition in liver and muscle, and decreased cardio-respiratory fitness. We further hypothesize that abnormalities along the "muscle axis" (intra-myocellular lipid content, insulin-mediated glucose disposal, and cardio-respiratory fitness) are more pronounced in MONW subjects of Indian descent, whereas abnormalities along the "visceral axis" (intra-hepatic fat content, intra-abdominal adipose tissue, and β-cell function) are more pronounced in MONW subjects of Chinese descent.

Hypothesis 2: We hypothesize that, compared with a control condition of weight maintenance (i.e. no change in diet and physical activity), diet-induced *mild* weight loss (5 % of initial body weight) reduces ectopic fat deposition and improves metabolic function in MONW subjects, whereas matched exercise-induced weight loss causes *greater*, and exercise without weight loss causes *smaller* beneficial effects in ectopic fat deposition and metabolic function.

To test these hypotheses, total body fat (dual-energy X-ray absorptiometry), intra-abdominal adipose tissue (magnetic resonance imaging), intra-myocellular and intra-hepatic lipid (magnetic resonance spectroscopy), insulin-mediated glucose disposal (hyperinsulinemic-euglycemic clamp), β-cell function (mixed meal tolerance test), and cardio-respiratory fitness (maximum oxygen consumption test) will be evaluated in metabolically healthy and unhealthy lean subjects of Indian and Chinese descents, matched for BMI and percent body fat (**Hypothesis 1**), and in MONW subjects before and after calorie restriction- or exercise- induced weight loss, exercise without weight loss, or weight maintenance (**Hypothesis 2**).

Results from this project are expected to result in the efficient targeting of health promotion programs in Singapore and the development of effective lifestyle modification approaches to reduce cardiometabolic disease risk in metabolically-unhealthy lean individuals. This will minimize healthcare expenditures, positively affect productivity, and ultimately improve the health and the quality of life of the Singaporean population, and inform public health programs in other countries.

BACKGROUND & CLINICAL SIGNIFICANCE

Obesity and type 2 diabetes in Singapore

The incidence of overweight and obesity has been increasing during the past 2–3 decades in Singapore, and is expected to increase further in the future [1]. By the year 2050, it is estimated that more than half of the population will be overweight or obese, defined as having a body mass index (BMI, calculated as the weight in kilograms divided by the square of height in meters) equal to or greater than 25 kg/m^2 [1]. This is likely responsible, at least in part, for the concomitant increase in obesity-related co-morbid conditions, particularly type 2 diabetes [1, 2]. The relationship between BMI and the risk for type 2 diabetes in populations from the Asia-Pacific region is linear within a wide range of BMI values (from ~21 kg/m^2 to ~34 kg/m^2), so that for every 2 kg/m^2 increase in BMI (which corresponds to ~6 kg for a normal-weight person of average stature), the risk for developing type 2 diabetes rises by ~27 % [2]. In Singapore, the prevalence of type 2 diabetes is expected to double from 7.3 % in 1990 to ~15 % in 2050, predominantly as a result of the fattening of the population, with the burden being greater for those of Indian descent than those of Chinese descent [1]. This is expected to reduce productivity, inflate healthcare costs, and increase mortality among Singaporeans [1, 3].

Metabolic dysfunction in normal-weight individuals

The prevalence of type 2 diabetes in Singapore is similar to that in the Unites States, even though the prevalence of overweight and obesity (BMI ≥25 kg/m^2) in Singapore is approximately half that in the US [4]. This observation corroborates findings from many studies showing that markers of metabolic dysfunction (e.g. hyperglycemia, hyperinsulinemia, insulin resistance, dyslipidemia, and hypertension) are highly prevalent among Singaporean adults even at normal BMI values, i.e. even among people who are considered "normal-weight" or "lean" by conventional measures [5, 6]. The existence of people who have normal body weight but also have metabolic dysfunction, and therefore greater risk for developing cardiometabolic disease, was recognized several decades ago [7, 8]. At the extreme of this paradigm, even among members of the Calorie Restriction Society who undergo self-imposed calorie restriction for years based on the belief that this will help them ensure a long and healthy life, there are many individuals (~40 %) with impaired glucose tolerance, despite very low BMI and total body fat [9]. These individuals are often referred to as "metabolically-obese normal-weight" (MONW) or "metabolically-abnormal lean" or "metabolically-unhealthy lean" subjects. The prevalence of this phenotype ranges from 5 % to 45 % depending on the BMI and the metabolic criteria used for its definition, as well as the characteristics of the population (i.e. age, sex, and ethnicity) [10, 11]. Similar variability has been observed across Asia [12-16]. For example, among the Chinese, ~8 % of the population as a whole, or ~13 % of those who are considered lean by virtue of body fat percent (i.e. ≤25 % for men and ≤35 % for women), are metabolically unhealthy, defined as having three or more metabolic abnormalities characteristic of the metabolic syndrome [13]. Among Indians, on the other hand, 15–25 % of the population (or 20–40 % of those who are considered lean by virtue of BMI, i.e. <25 kg/m^2) satisfies the criteria for metabolic syndrome [16, 17]. The MONW phenotype in Asians is associated with 3-fold greater risk for carotid atherosclerosis (i.e. cardiovascular disease) [14] and 4.5–8.5-fold greater risk for developing type 2 diabetes [13]. In fact, MONW subjects have increased risk for cardiometabolic disease [13, 14] and greater all-cause mortality [18] not only compared to *metabolically-healthy lean* subjects, but also compared to *metabolically-healthy obese* subjects. This underscores the importance of metabolic dysfunction independent of excess body weight and total adiposity.

The MONW phenotype

The mechanisms responsible for the development of metabolic abnormalities in lean people are not entirely clear. The MONW phenotype can manifest early in life, e.g. during childhood [19], which corroborates the existence of genetic predisposition for metabolic dysfunction in the face of low BMI values [20]. Previous studies have identified a number of factors associated with the MONW phenotype, including increased intra-abdominal (visceral) adipose tissue, increased liver and muscle fat content, increased fat cell size, adipose tissue inflammation, altered inflammatory and adipokine profiles, reduced skeletal muscle mass, lack of physical activity, and low cardio-respiratory fitness [7, 9-11, 13, 16, 21-29]. All of these factors have been directly or indirectly associated with insulin resistance (defined by a variety of methods), which is by far the commonest metabolic correlate of the MONW phenotype across all ethnicities, age groups, and sexes [7, 10, 24, 27]. In fact, the greater prevalence of the MONW phenotype in Indians [16, 17] than in the Chinese [13] mirrors results obtained recently by our team, showing

that among *lean* Singaporean men (BMI <25 kg/m^2 or body fat ≤20 %), those of Indian descent have significantly lower insulin sensitivity, evaluated as the insulin-mediated glucose disposal rate during a hyperinsulinemic-euglycemic clamp procedure, compared to those of Chinese descent [30]. Similar results have been reported by other investigators in smaller groups of subjects [31] or when using simpler indices of insulin sensitivity [32, 33]. Therefore, an insulin resistant glucose metabolism, broadly defined by subnormal responses to physiological insulin concentrations [34], is the hallmark of the MONW phenotype.

Owing to the lack of a consistent definition, there is some variability among studies in the phenotypic characterization of MONW subjects [11]. This is further complicated by the small but significant differences in BMI and, more commonly, percent body fat between groups of metabolically healthy and unhealthy lean subjects, with MONW subjects being always somewhat "fatter" (even though within the "lean" range) [7, 13, 16, 21-23, 28, 29]. Likewise, BMI and body fat are typically greater in relatively insulin-resistant (e.g. Indian) than in relatively insulin-sensitive (e.g. Chinese) individuals in studies reporting on ethnic differences in insulin action among lean people [30, 32]. This in itself could be responsible for the differences observed in metabolic function. There is considerable (~2-fold range) variability between individuals in the percent body fat [35, 36] and the insulin-mediated glucose disposal rate (a direct measure of whole-body insulin sensitivity) [37] for the same BMI value within the normal-weight range (i.e. BMI <25 kg/m^2), so that people with the same BMI can have very different body fat and insulin sensitivity without this necessarily being associated with the presence or absence of generalized metabolic dysfunction. Even among lean and metabolically-healthy Asians, total body fat is a major correlate of insulin-mediated glucose disposal [38]. It is thus possible that some of the reported differences between metabolically healthy and unhealthy lean subjects arise from normal variability and the differences in body fat between groups, rather than being an inherent characteristic of the MONW phenotype. In support of this possibility, when metabolically healthy and unhealthy lean subjects (defined as those having normal and impaired glucose tolerance, respectively) were retrospectively matched on total body fat, there were no differences between phenotypes in circulating concentrations of metabolic and inflammatory markers (i.e. high-density lipoprotein (HDL)-cholesterol, triglycerides, free fatty acids, C-reactive protein, adiponectin, and leptin) [9]. There are no studies that prospectively recruited groups of metabolically healthy and unhealthy lean individuals matched on BMI *and* percent body fat. A deeper understanding of the MONW phenotype, as proposed here, is important to dissect the metabolic abnormalities that are inherent to the phenotype from those merely associated with differences in total body fat. This will allow for proper identification and more efficient therapeutic targeting of MONW individuals, who are at greater risk for cardiometabolic disease.

Weight loss and metabolic function
Little is known about possible interventions for improving metabolic function in MONW subjects. It is well established that diet-induced weight loss can improve body composition and many of the cardiometabolic abnormalities in most obese patients (e.g. decreases total body fat, intra-abdominal adipose tissue, and ectopic fat deposition in liver and muscle; increases insulin sensitivity; improves blood lipid profile; and reduces blood pressure) [39-48], so that a *moderate* 10 % weight loss has become the cornerstone of obesity treatment [49]. However, MONW individuals are by definition lean, so recommending even moderate amounts of weight loss may not be a feasible therapeutic target [50]. It is therefore important to better understand the metabolic effects of smaller amounts of weight loss. Recently, we conducted a randomized controlled trial to evaluate the effects of *mild* weight loss (5 % of initial body weight) on cardiometabolic function in non-Asian subjects with obesity and insulin resistance, and found that even this small amount of weight loss decreases fat deposition in the liver and the intra-abdominal area, and increases insulin action in skeletal muscle, liver, and adipose tissue [51]. These results demonstrate that mild weight loss can improve many cardiometabolic abnormalities in metabolically-unhealthy *obese* subjects, but whether the same holds true for metabolically-unhealthy *lean* subjects is not known. A small, non-randomized, single-arm study in 7 lean, insulin-resistant offspring of parents with type 2 diabetes reported that modest ~6 % diet-induced weight loss reduced intra-myocellular lipid (i.e. fat within skeletal muscle fibers) content and increased insulin-mediated glucose disposal rate (both by ~30 % compared with baseline values), but did not significantly affect intra-abdominal adipose tissue volume or liver fat content [52]. It is thus not known whether mild diet-induced weight loss produces similar changes in body composition, fat distribution, and metabolic function in lean versus obese metabolically unhealthy subjects.

Endurance exercise and metabolic function

Exercise is a very potent intervention that can readily improve metabolic function, particularly insulin sensitivity [53-55], and when performed under controlled conditions, can lead to weight loss and favorable changes in body composition [56-58]. For the same amount weight loss (8–9 % of initial body weight) induced by a low-calorie diet or endurance exercise, exercise causes a greater reduction in fat mass, minimizes the decrease in muscle mass, and increases cardio-respiratory fitness compared with calorie restriction [56]. Also, exercise-induced weight loss results in a greater reduction in intra-abdominal adipose tissue (by ~20 %) [56], a greater increase in insulin-mediated glucose disposal during a hyper-insulinemic-euglycemic clamp (by ~30 %) [56], and a much greater reduction in the total insulin response to an oral glucose tolerance test (by ~2.5–fold) [57] compared with matched diet-induced weight loss, although these differences did not reach statistical significance. These observations raise the possibility that exercise may be more advantageous than calorie restriction for improving metabolic function in MONW subjects. This is an intriguing hypothesis given that the MONW phenotype is frequently associated with low levels of habitual physical activity, reduced muscle mass, and low cardio-respiratory fitness [9, 22, 25, 26, 28, 29].

Knowledge gaps fulfilled by this project

Our understanding of the complex interactions between ethnicity, body composition, and metabolic dysfunction and its reversal by calorie restriction and exercise remains rudimentary. Accordingly, a better understanding of the MONW phenotype and the evaluation of the efficacy of different therapeutic approaches for its reversal will have important implications for public health, particularly in a multi-ethnic population, because it will help identify and intervene earlier in these subjects who are more likely to go undiagnosed and thus less likely to be treated before clinically overt cardiometabolic disease develops. To this end, this study aims to test the following hypotheses:

Hypothesis 1: We hypothesize that, compared with metabolically-healthy lean subjects, MONW subjects have reduced insulin-mediated glucose disposal, impaired β-cell function, increased intra-abdominal adipose tissue, more ectopic fat deposition in liver and muscle, and decreased cardio-respiratory fitness. We further hypothesize that abnormalities along the "muscle axis" (intra-myocellular lipid content, insulin-mediated glucose disposal, and cardio-respiratory fitness) are more pronounced in MONW subjects of Indian descent, whereas abnormalities along the "visceral axis" (intra-hepatic fat content, intra-abdominal adipose tissue, and β-cell function) are more pronounced in MONW subjects of Chinese descent.

Hypothesis 2: We hypothesize that, compared with a control condition of weight maintenance (i.e. no change in diet and physical activity), diet-induced *mild* weight loss (5 % of initial body weight) reduces ectopic fat deposition and improves metabolic function in MONW subjects, whereas matched exercise-induced weight loss causes *greater*, and exercise without weight loss causes *smaller* beneficial effects in ectopic fat deposition and metabolic function.
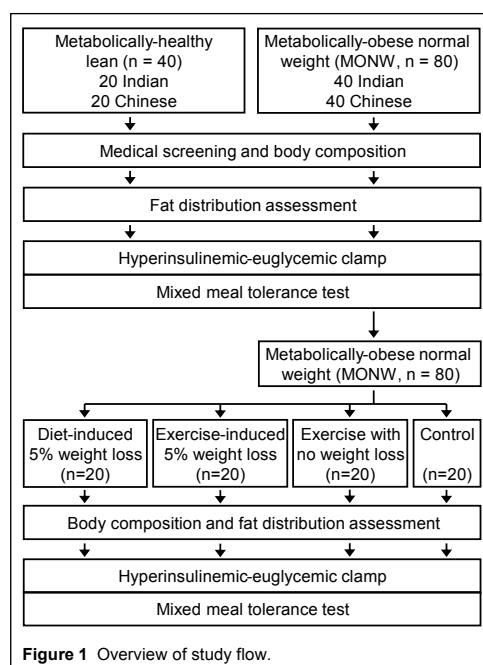
METHODS / APPROACH

Subjects

A total of 120 men and women between 21 and 65 yrs old will participate in this study: 40 metabolically-healthy lean subjects (20 Indian and 20 Chinese) and 80 metabolically-obese, normal-weight (MONW) subjects (40 Indian and 40 Chinese). The two groups will be balanced on age, sex, ethnicity, BMI and percent body fat, to avoid confounding metabolic function outcomes due to differences in these factors. Matching for BMI and body fat is particularly important to circumvent the limitation in interpreting the results from previous studies in which MONW subject groups were "fatter" than the respective control groups of metabolically-healthy lean subjects [7, 21, 28]. As insulin resistance is the hallmark of the MONW phenotype [7, 10, 24, 27], we will use ethnicity- and sex- specific cutoff values [33] of the homeo-stasis model assessment of insulin resistance (HOMA-IR) score, calculated from fasting plasma glucose and insulin concentrations [59], to classify subjects as metabolically healthy or MONW at screening. HOMA-IR scores >1.41 for Chinese men and women, and >2.17 and >1.99 for Indian men and women, respectively, will be considered indicative of metabolic dysfunction; these values correspond to the upper limit of the 95 % confidence interval of HOMA-IR (i.e. the most insulin resistant) in Singaporeans with normal glucose tolerance [33].

Based on the prevalence of the MONW phenotype in the Chinese and the Indians [13, 16, 17], who constitute ~85 % of the Singaporean population, we estimate we will need to screen ~650 individuals to identify 80 MONW and 40 metabolically-healthy subjects. We do not anticipate any difficulties in screening and recruiting the required number of subjects for this study. We intend to post flyers and disseminate information about the study to a large number of potential volunteers through our diabetes, lipid, and cardiometabolic clinics at the National University Health System Hospitals and general endocrinology clinics at the Tan Tock Seng Hospital. This effort will be complemented by dissemination of information about the study through blast emails to students and employees at the National University of Singapore (NUS) and the Nanyang Technological University (NTU), posted flyers, and word-of-mouth. Written informed consent will be obtained from each subject before participation in the study. During the screening visit at the CNRC, subjects will complete a comprehensive medical evaluation, including a history and physical examination, and standard blood tests. They will also have their body fat percent measured by using dual-energy X-ray absorptiometry (DEXA). Women of child-bearing potential will have a urine pregnancy test. The following exclusion criteria will be used: i) BMI ≥25 kg/m^2, but also <19 kg/m^2 (to avoid the risk of subjects becoming seriously underweight (i.e. BMI ≤18 kg/m^2) after 5 % weight loss); ii) age <21 and >65 yrs; iii) use of medications that can affect metabolic function (including oral contraceptives and hormone replacement therapy); iv) regular use of tobacco products; v) regular consumption of alcohol; vi) pregnant or breastfeeding women; vii) evidence of significant organ system dysfunction or disease; viii) recent weight loss (≥5 % over the past 6 months); and ix) severe asthma and respiratory problems that prevent subjects from exercising.

Experimental design

An overview of the study flow is shown in Figure 1. This project has a cross-sectional arm (observational study to evaluate differences between MONW and metabolically-healthy lean subjects from two different ethnic backgrounds; **Hypothesis 1**) and an intervention arm (randomized controlled trial to assess the effects of calorie restriction and exercise in MONW subjects; **Hypothesis 2**). Inclusion of a metabolically-healthy lean group is important for the unbiased characterization of the MONW phenotype (by comparing results for MONW subjects against a sex-, age-, ethnicity-, BMI- and percent body fat- matched group of metabolically-healthy lean people), but also for the better evaluation of the effects of lifestyle intervention in MONW subjects (by comparing results after calorie restriction and exercise not only against values at baseline, but also against the "normal" range of values for lean people). All outcomes will be assessed in exactly the same manner one time in metabolically-healthy lean subjects and two times in MONW subjects (once at baseline and again after the interventions), after making sure that participants have remained weight-stable for at least two weeks (≤1 % change in body weight/wk; both at baseline and after the interventions) to avoid potential confounding of our measures from acute changes in energy balance. Following completion of baseline testing, MONW subjects will be randomly assigned to one of four intervention groups (n = 20 in each; 10 Indian and 10 Chinese): *i*) hypocaloric diet to induce 5 % weight loss, *ii*) endurance exercise without changes in energy intake (i.e. isocaloric diet) to induce 5 % weight loss, *iii*) endurance exercise with a compensatory increase in energy intake to maintain body weight, or *iv*) no change in exercise and diet to maintain body weight (i.e. control).

Figure 1 Overview of study flow.

Following the successful screening and enrollment into the study, subjects will visit the CNRC and CIRC (both facilities are located in the same building at NUS campus) to have their resting metabolic rate, cardio-respiratory fitness, and body fat distribution assessed. Two weeks later (diet lead-in period, during which the energy requirements for weight maintenance will be tailored for each subject), and provided body weight has remained stable (≤1 % change per week), subjects will visit the CNRC on two additional occasions, separated by 5–10 days, to undergo: *i*) a 3-hour hyper-insulinemic-euglycemic clamp procedure, to determine whole-body insulin-mediated glucose disposal and insulin sensitivity [30], and *ii*)

a 3-hour mixed meal tolerance test (MMTT), to determine glucose tolerance, insulin secretion, and β-cell function [60-62]. This battery of tests combines advanced medical imaging techniques to assess the amount of adipose tissue and its distribution in the body, with state-of-the-art (i.e. glucose uptake during infusion of insulin) as well as physiological (i.e. responses to meal ingestion) tests to assess metabolic function, thereby allowing for a deep and comprehensive phenotypic characterization of our subjects.

Resting energy expenditure and cardio-respiratory fitness. Subjects will visit the CNRC in the morning, after having fasted overnight, and having abstained from strenuous exercise, alcohol, and caffeine on the previous day. Following 30 min of bed rest, they will have their oxygen consumption and carbon dioxide production measured continuously for 30 min, while they breathe under a ventilated hood, and resting metabolic rate (RMR) will be determined by using indirect calorimetry (Quark RMR; COSMED, Rome, Italy) [63]. Thereafter, they will undergo a graded exercise test (standard Bruce protocol [64, 65]) on the treadmill (Pro+; Cybex International, Medway, MA), while breathing through a face mask, to determine their maximal oxygen consumption (VO_{2max}) (Metanalyzer 3B; CORTEX Biophysik GmbH, Leipzig, Germany). This test involves increasing the treadmill speed and grade every 3 minutes (to allow achievement of a steady state) until volitional exhaustion.

Imaging for body composition. Fat mass and fat-free mass will be determined by using dual-energy X-ray absorptiometry (DEXA) on a Discovery QDR Series DEXA scanner (Hologic, Bedford, MA, USA) in the CNRC. Intra-abdominal adipose tissue (visceral fat) and subcutaneous abdominal adipose tissue volumes will be determined by using magnetic resonance (MR) imaging. Intra-hepatic triglyceride (IHTG) and intra-myocellular lipid (IMCL) contents will be determined by using proton MR spectroscopy. MR imaging and spectroscopy will be conducted on a 3 Tesla MR scanner (Tim Trio, Siemens, Erlangen, Germany) in the CIRC; details of the scanning and analysis procedures have been described in detail previously [30].

Metabolic testing
For both metabolic testing visits (clamp and MMTT), which will be scheduled ~1 wk apart, subjects will come to the CNRC in the morning, after having fasted overnight. They will be instructed to abstain from alcohol and caffeine consumption on the previous day, and from performing any strenuous exercise on the preceding 3 days (to avoid potential delayed metabolic effects of exercise). Subjects will use food diaries given to them at screening to record all food and drinks consumed on the day before they come to the CNRC for their first metabolic testing visit, and will be instructed to replicate the same diet on the day preceding the remaining visits. On all occasions, vital signs (temperature, heart rate, and blood pressure) will be obtained after 30 min of bed rest, and before any testing begins.

Hyperinsulinemic-euglycemic clamp. Whole-body insulin sensitivity will be determined by using a 3-hour hyperinsulinemic-euglycemic clamp [30, 66]. Indwelling catheters will be inserted into an antecubital vein of one arm to infuse insulin and dextrose, and a forearm vein in the contralateral arm for blood sampling. After a baseline fasting blood sample is obtained, insulin will be infused at a constant rate of 50 mU/m^2 body surface area/min for the duration of the clamp (180 min). Plasma glucose concentration will be measured every 10 min at bedside and the infusion rate of 20 % dextrose solution will be adjusted accordingly to maintain euglycemia (100 mg/dL or 5.6 mmol/L). At the beginning of this test, a muscle biopsy with be performed, which involves obtaining a small piece (the size of a corn kernel) of leg muscle (quadriceps) under sterile conditions and local anesthesia, to evaluate cellular factors involved in glucose handling by muscle.

Mixed meal tolerance test (MMTT). An indwelling catheter will be inserted into a forearm vein of one arm to obtain blood samples. After a baseline fasting blood sample is obtained, a 600-kcal liquid mixed meal (55 % of energy from carbohydrate, 15 % from protein, and 30 % from fat) will be consumed over 5 minutes (time will start counting at the beginning of the meal). Blood samples to measure glucose, C-peptide, and insulin concentrations will be collected according to the following schedule: 10, 20, 30, 45, 60, 90, 120, 150, and 180 minutes.

Diet and exercise interventions

Daily energy requirements for weight maintenance (i.e. isocaloric diet) will be estimated for each subject by multiplying resting energy expenditure (i.e. RMR) by a factor of 1.4 [67]. All MONW subjects will be prescribed an isocaloric diet for the 2 weeks preceding their first metabolic testing visit (diet lead-in period). During this time, all food will be prepared in the metabolic kitchen of the CNRC and will be provided to all subjects, to familiarize them with food portions at the prescribed energy intake. Body weight will be monitored and the energy content of the diet will be adjusted accordingly to maintain weight (≤1 % change per week). This will ensure a fine-tuned, tailored determination of energy requirements for weight maintenance. Thereafter, MONW subjects will be randomized to one of four intervention groups consisting of different manipulations of diet and exercise (Figure 2).

	Control	Diet-induced weight loss	Exercise-induced weight loss	Exercise without weight loss
Diet (energy intake)	↔	↓	↔	↑
Exercise (energy expenditure)	↔	↔	↑	↑
Expected weight change	↔	↓	↓	↔

Figure 2 Diet and exercise interventions and expected weight changes in MONW subjects randomized in different intervention groups.

MONW subjects randomized in the diet and exercise weight loss groups will participate in a supervised weight loss program to help ensure they are under a similar weekly energy deficit and achieve a 5 % weight loss at approximately the same time. Participants in the *diet-induced weight loss* group (n = 20) will be prescribed a reduced-calorie diet (~500 kcal/d below their needs for weight maintenance), and will be instructed not to change their physical activity habits, in order to achieve a weekly weight loss of ~0.5 kg. Participants in the *exercise-induced weight loss* group (n = 20) will be prescribed an isocaloric diet and an endurance exercise program designed to elicit an energy deficit of ~500 kcal/d, to achieve the same weekly weight loss of ~0.5 kg. Participants in the *exercise without weight loss* group (n = 20) will be prescribed the same endurance exercise program but their caloric intake will be increased accordingly to prevent any changes in body weight. Finally, participants in the *control* group (n = 20) will be instructed to maintain their physical activity habits and consume an isocaloric diet to maintain their body weight.

The macronutrient composition of the diet will be the same for all groups (55–60 % of energy from carbohydrate, 15–20 % from protein, and 20–30 % from fat); no vitamins or other nutritional supplements will be given. The prescribed diet and exercise programs will be individualized based on each subject's food and exercise preferences. During the first week of the intervention, all food will be provided to subjects in the diet-induced weight loss group and the exercise without weight loss group, to ensure they get accustomed to the new prescribed energy intake (reduced and increased relative to their isocaloric needs, respectively). Likewise, all exercise sessions for subjects in the exercise-induced weight loss group and the exercise without weight loss group will be supervised, to ensure they get familiarized with the exercise workload and the different options for exercise mode, intensity, and duration. A member of the research team will be contacting the subjects on a weekly basis to monitor progress, foster adherence to diet and exercise prescription, identify possible problems, and build a supportive environment for the participant.

Sample analyses
Plasma glucose concentration will be determined by using the glucose oxidase method on an automated glucose analyzer (YSI 2300 Stat Plus; YSI Life Sciences, Yellow Spring, OH, USA). Plasma insulin and C-peptide concentrations will be determined by using electrochemiluminescence technology (Roche/Hitachi cobas e411 immunochemistry analyzer; Roche Diagnostics, Indianapolis, IN). Total plasma triglyceride and HDL-cholesterol concentrations will be determined by using a colorimetric and an immuno-turbidimetric assay, respectively, on an automated analyzer (Roche/Hitachi cobas c311 clinical chemistry analyzer; Roche Diagnostics, Indianapolis, IN). Plasma concentrations of leptin, adiponectin, C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) will be measured by using commercially available enzyme-linked immunosorbent assay (ELISA) kits from R&D Systems (Minneapolis, MN, USA), ALPCO Diagnostics (Salem, NH, USA), and EMD Millipore (Billerica, MA, USA).

Calculations

Insulin sensitivity. The average rate of dextrose infusion during the final 30 minutes of the clamp corresponds to the insulin-mediated whole-body glucose disposal (M value). Insulin sensitivity will be calculated as the M/I ratio, i.e. glucose disposal divided by the steady-state plasma insulin concentration (i.e. average insulin concentration during the final 30 minutes of the clamp) [30]. The M/I ratio adjusts glucose disposal rate for potential differences in insulin concentrations attained during the clamp.

β-Cell function. The insulin secretion rate (ISR) response sensitivity to plasma glucose after ingestion of the mixed meal will be assessed by using oral minimal model analysis (SAAM II version 2) of plasma C-peptide concentrations, to obtain Φ-static (ISR response sensitivity to plasma glucose concentration), Φ-dynamic (ISR response sensitivity to the rate of change in plasma glucose concentration), and Φ-total (overall ISR response sensitivity to plasma glucose) [60-62]. The disposition index, which provides an assessment of the appropriateness of insulin secretion in relationship to peripheral insulin sensitivity, will be determined as the product of insulin sensitivity and insulin secretion [68, 69].

Glucose tolerance. Oral glucose tolerance will be determined by integrating (with the trapezoid rule) the area under the glucose concentration vs. time curve (AUC) for 3 hours after ingestion of the mixed meal.

Statistical Analysis

General principles. Prior to all statistical analyses, the distributional properties of the outcome measures will be evaluated for normality (Shapiro-Wilks test) and equal variance (Levene's test). If the variables are not appropriately distributed, data transformations will be utilized to produce distributions that satisfy normality and equal variance assumptions. If an appropriate transformation cannot be found, semi-parametric methods based on the ranks of the data will be used; otherwise, parametric tests will be used. In the cross-sectional arm of the study, the primary goal will be to determine whether results obtained in MONW subjects differ from those obtained in metabolically-healthy subjects, and whether the differences between metabolic groups are modulated by ethnic background (Indian or Chinese) (**Hypothesis 1**). These goals will be accomplished by using analysis of variance with two between-subject factors. The main effects of metabolic group and ethnicity, as well as the interaction between these two factors will be determined. Statistically significant interactions, indicating that differences between metabolically healthy and unhealthy subjects vary by ethnicity, will be followed by Tukey's post-hoc tests within each ethnic group. In the interventional arm of the study, the primary goal will be to determine whether the changes induced by calorie restriction and exercise in the three intervention groups differ from the corresponding changes in the control group, as well as from each other (**Hypothesis 2**). This will be accomplished by using repeated measures analysis of covariance, with the post-intervention value as the dependant variable, the intervention group as the independent variable, and the baseline value as a covariate. In a secondary analysis, we will examine whether ethnicity modulates the response to diet or exercise, by using ethnic background (Indian or Chinese) as an additional between-subject factor. This will be an *exploratory aim*, given our study is not adequately powered to address this question (we would need twice as many subjects in the intervention arm). Nevertheless, out of the 20 subjects in each intervention group, we will recruit 10 from each ethnicity, and this will likely be enough to provide an indication of an ethnic-specific response to calorie restriction and exercise (if indeed any). All these analyses will be preceded by preliminary between-group comparisons using Student's unpaired t test (or the Mann-Whitney U test), and χ^2 test, to confirm there are no differences between groups with respect to key prognostic and demographic variables at baseline. Although unlikely, given the planned balancing of the groups, any such variables that differ between groups at baseline will be adjusted for in subsequent analyses of covariance. The fit of all models will be evaluated by assessing regression residuals. We will accept statistical significance (type I error) at $P \leq 0.05$. Statistical analysis will be performed by using SPSS version 23 (IBM SPSS, Chicago, IL).

Sample size considerations. Our primary endpoint is whole-body insulin sensitivity (i.e. the major metabolic correlate of the MONW phenotype), determined by using the hyperinsulinemic-euglycemic clamp. Previous studies that evaluated insulin-mediated glucose disposal in groups of healthy lean subjects on multiple occasions reported intra-individual coefficients of variation (CV) of 3–22 % [70-72]. Under a conservative scenario, i.e. intra-individual CV of 21 % for insulin sensitivity, and an expected difference between metabolically healthy and unhealthy subjects in the order of ~25 % [22, 73], and an expected effect of mild weight loss in the order of ~20 % [51], we estimated we would need 14 subjects per group in the cross-sectional arm, and 9 subjects per group in the interventional arm, to detect statistically significant differences of this magnitude between metabolically healthy and unhealthy subjects

and before and after the interventions, respectively, using two-sided tests at the 5 % level of significance (i.e. type I error, α = 0.05) and with 80 % power (i.e. type II error, β = 0.20) [74]. Given that both of our hypotheses ultimately involve comparing different groups of people (whether comparing data at one point in time between two groups, as in **Hypothesis 1**; or comparing the change occurring between two points in time among four groups, as in **Hypothesis 2**), we decided to recruit 20 subjects per group in both the cross-sectional and the interventional arms of the study based on another conservative assumption, i.e. that only 70 % of all subjects will complete the study (n = 14 per group; dropout rate of 30%). Based on our experience with similar lifestyle interventions leading to small amounts of weight loss [51], we expect much lower dropout rates (<25 %), so we are confident our study has more than adequate statistical power to meet its primary goals.

Special considerations
Definition of metabolic dysfunction. Insulin resistance is the key metabolic abnormality of the MONW phenotype [7, 10, 24, 27], so the HOMA-IR score will be utilized to classify subjects as metabolically healthy or unhealthy at screening. One might therefore expect that the hypothesized differences between groups in the M value and M/I ratio (Hypothesis 1) will be significant by definition. However, the HOMA-IR score is calculated from fasting plasma glucose and insulin concentrations [59] and is only a crude index that is not synonymous to (although it does correlate with) insulin sensitivity determined by the hyperinsulinemic-euglycemic clamp. It may actually be more reflective of hepatic insulin sensitivity [75], whereas insulin-mediated glucose disposal determined during the clamp procedure is more reflective of muscle insulin sensitivity. In addition, the cutoff values that will be used are based on the relationship between HOMA-IR and glucose tolerance [33], which is a function of both insulin sensitivity and insulin secretion. These lines of reasoning support the argument that differences in HOMA-IR between groups (by design) cannot be considered equivalent to differences in insulin sensitivity, insulin secretion, or both.
Definition of normal-weight. The choice of a BMI <25 kg/m^2 to indicate "leanness" may not be optimal for the Singaporean population, because of a varying relationship between BMI and percent body fat in Asian and Caucasian populations, but also among Asian populations [76, 77]. This implies that some subjects in the high-normal BMI range may actually be "overweight" by Asian standards. Nevertheless, this will not affect interpretation of our results because metabolically healthy and unhealthy subjects will be matched for both BMI and percent body fat in this study.
Subject retention. No major problems with subject retention are expected, given the relatively modest intensity and short duration of the interventions. Based on our experience [51], we anticipate 20-25 % of subjects will drop out or fail to comply with prescribed diet and exercise regimens. However, the sample size was calculated based on a 30 % dropout rate to ensure an adequate number of subjects complete the study, even in the event of unanticipated greater dropout rates.
Safety of study subjects. This study involves consuming a low calorie diet or performing endurance exercise for 13-15 weeks. In addition, subjects will undergo complex metabolic studies before and after the interventions. Our research team has considerable experience in conducting exactly these types of studies, and has the clinical expertise to provide careful medical monitoring throughout the study to reduce the risk of any medical problems. The research team will meet every week to review progress, problems and any clinical issues that arise during the study and either Dr. Leow or Dr. Toh will be available 24/7 on an on-call basis to address any medical problems that require urgent attention. Also, we carefully designed the metabolic studies to ensure that the total amount of blood drawn from all research procedures is ~250 mL before and ~250 mL after the interventions, over a 4-month period.

REFERENCES

1   Phan TP, Alkema L, Tai ES, Tan KH, Yang Q, Lim WY, Teo YY, Cheng CY, Wang X, Wong TY, Chia KS, Cook AR. Forecasting the burden of type 2 diabetes in Singapore using a demographic epidemiological model of Singapore. BMJ Open Diabetes Res Care 2014; **2:** e000012.

2   Ni Mhurchu C, Parag V, Nakamura M, Patel A, Rodgers A, Lam TH, Asia Pacific Cohort Studies Collaboration. Body mass index and risk of diabetes mellitus in the Asia-Pacific region. Asia Pac J Clin Nutr 2006; **15:** 127-133.

3   Ma S, Cutter J, Tan CE, Chew SK, Tai ES. Associations of diabetes mellitus and ethnicity with mortality in a multiethnic Asian population: data from the 1992 Singapore National Health Survey. Am J Epidemiol 2003; **158:** 543-552.

4   Yoon KH, Lee JH, Kim JW, Cho JH, Choi YH, Ko SH, Zimmet P, Son HY. Epidemic obesity and type 2 diabetes in Asia. Lancet 2006; **368:** 1681-1688.

5   Deurenberg-Yap M, Yian TB, Kai CS, Deurenberg P, WA VANS. Manifestation of cardiovascular risk factors at low levels of body mass index and waist-to-hip ratio in Singaporean Chinese. Asia Pac J Clin Nutr 1999; **8:** 177-183.

6   Deurenberg-Yap M, Chew SK, Lin VF, Tan BY, van Staveren WA, Deurenberg P. Relationships between indices of obesity and its co-morbidities in multi-ethnic Singapore. Int J Obes Relat Metab Disord 2001; **25:** 1554-1562.

7   Ruderman N, Chisholm D, Pi-Sunyer X, Schneider S. The metabolically obese, normal-weight individual revisited. Diabetes 1998; **47:** 699-713.

8   Ruderman NB, Schneider SH, Berchtold P. The "metabolically-obese," normal-weight individual. Am J Clin Nutr 1981; **34:** 1617-1621.

9   Fontana L, Klein S, Holloszy JO. Effects of long-term calorie restriction and endurance exercise on glucose tolerance, insulin action, and adipokine production. Age (Dordr) 2010; **32:** 97-108.

10   Conus F, Rabasa-Lhoret R, Peronnet F. Characteristics of metabolically obese normal-weight (MONW) subjects. Appl Physiol Nutr Metab 2007; **32:** 4-12.

11   Teixeira TF, Alves RD, Moreira AP, Peluzio Mdo C. Main characteristics of metabolically obese normal weight and metabolically healthy obese phenotypes. Nutr Rev 2015; **73:** 175-190.

12   Lee SH, Ha HS, Park YJ, Lee JH, Yim HW, Yoon KH, Kang MI, Lee WC, Son HY, Park YM, Kwon HS. Identifying metabolically obese but normal-weight (MONW) individuals in a nondiabetic Korean population: the Chungju Metabolic disease Cohort (CMC) study. Clin Endocrinol (Oxf) 2011; **75:** 475-481.

13   Luo D, Liu F, Li X, Yin D, Lin Z, Liu H, Hou X, Wang C, Jia W. Comparison of the effect of 'metabolically healthy but obese' and 'metabolically abnormal but not obese' phenotypes on development of diabetes and cardiovascular disease in Chinese. Endocrine 2015; **49:** 130-138.

14   Yoo HJ, Hwang SY, Hong HC, Choi HY, Seo JA, Kim SG, Kim NH, Choi DS, Baik SH, Choi KM. Association of metabolically abnormal but normal weight (MANW) and metabolically healthy but obese (MHO) individuals with arterial stiffness and carotid atherosclerosis. Atherosclerosis 2014; **234:** 218-223.

15   Jung CH, Lee MJ, Kang YM, Jang JE, Leem J, Hwang JY, Kim EH, Park JY, Kim HK, Lee WJ. The risk of incident type 2 diabetes in a Korean metabolically healthy obese population: the role of systemic inflammation. J Clin Endocrinol Metab 2015; **100:** 934-941.

16   Indulekha K, Surendar J, Anjana RM, Geetha L, Gokulakrishnan K, Pradeepa R, Mohan V. Metabolic obesity, adipocytokines, and inflammatory markers in Asian Indians--CURES-124. Diabetes Technol Ther 2015; **17:** 134-141.

17   Geetha L, Deepa M, Anjana RM, Mohan V. Prevalence and clinical profile of metabolic obesity and phenotypic obesity in Asian Indians. J Diabetes Sci Technol 2011; **5:** 439-446.

18   Choi KM, Cho HJ, Choi HY, Yang SJ, Yoo HJ, Seo JA, Kim SG, Baik SH, Choi DS, Kim NH. Higher mortality in metabolically obese normal-weight people than in metabolically healthy obese subjects in elderly Koreans. Clin Endocrinol (Oxf) 2013; **79:** 364-370.

19   Guerrero-Romero F, Aradillas-Garcia C, Simental-Mendia LE, Torres-Rodriguez ML, Mendoza Ede L, Rosales-Cervantes J, Rodriguez-Ramirez G, Rodriguez-Moran M. Biochemical characteristics and risk factors for insulin resistance at different levels of obesity. Pediatrics 2013; **131:** e1211-1217.

20   Yaghootkar H, Scott RA, White CC, Zhang W, Speliotes E, Munroe PB, Ehret GB, Bis JC, Fox CS, Walker M, Borecki IB, Knowles JW, Yerges-Armstrong L, Ohlsson C, Perry JR, Chambers JC, Kooner JS, Franceschini N, Langenberg C, Hivert MF, Dastani Z, Richards JB, Semple RK, Frayling TM. Genetic evidence for a normal-weight "metabolically obese" phenotype linking insulin resistance, hypertension, coronary artery disease, and type 2 diabetes. Diabetes 2014; **63:** 4369-4377.

21   Badoud F, Perreault M, Zulyniak MA, Mutch DM. Molecular insights into the role of white adipose tissue in metabolically unhealthy normal weight and metabolically healthy obese individuals. FASEB J 2015; **29:** 748-758.

22   Dvorak RV, DeNino WF, Ades PA, Poehlman ET. Phenotypic characteristics associated with insulin resistance in metabolically obese but normal-weight young women. Diabetes 1999; **48:** 2210-2214.

23   De Lorenzo A, Del Gobbo V, Premrov MG, Bigioni M, Galvano F, Di Renzo L. Normal-weight obese syndrome: early inflammation? Am J Clin Nutr 2007; **85:** 40-45.

24   Karelis AD, St-Pierre DH, Conus F, Rabasa-Lhoret R, Poehlman ET. Metabolic and body composition factors in subgroups of obesity: what do we know? J Clin Endocrinol Metab 2004; **89:** 2569-2575.

25   Kim TN, Park MS, Yang SJ, Yoo HJ, Kang HJ, Song W, Seo JA, Kim SG, Kim NH, Baik SH, Choi DS, Choi KM. Body size phenotypes and low muscle mass: the Korean sarcopenic obesity study (KSOS). J Clin Endocrinol Metab 2013; **98:** 811-817.

26   Lee K. Metabolically obese but normal weight (MONW) and metabolically healthy but obese (MHO) phenotypes in Koreans: characteristics and health behaviors. Asia Pac J Clin Nutr 2009; **18:** 280-284.

27   Oliveros E, Somers VK, Sochor O, Goel K, Lopez-Jimenez F. The concept of normal weight obesity. Prog Cardiovasc Dis 2014; **56:** 426-433.

28   Di Renzo L, Del Gobbo V, Bigioni M, Premrov MG, Cianci R, De Lorenzo A. Body composition analyses in normal weight obese women. Eur Rev Med Pharmacol Sci 2006; **10:** 191-196.

29   Conus F, Allison DB, Rabasa-Lhoret R, St-Onge M, St-Pierre DH, Tremblay-Lebeau A, Poehlman ET. Metabolic and behavioral characteristics of metabolically obese but normal-weight women. J Clin Endocrinol Metab 2004; **89:** 5013-5020.

30   Khoo CM, Leow MK, Sadananthan SA, Lim R, Venkataraman K, Khoo EY, Velan SS, Ong YT, Kambadur R, McFarlane C, Gluckman PD, Lee YS, Chong YS, Tai ES. Body fat partitioning does not explain the interethnic variation in insulin sensitivity among Asian ethnicity: the Singapore adults metabolism study. Diabetes 2014; **63:** 1093-1102.

31   Liew CF, Seah ES, Yeo KP, Lee KO, Wise SD. Lean, nondiabetic Asian Indians have decreased insulin sensitivity and insulin clearance, and raised leptin compared to Caucasians and Chinese subjects. Int J Obes Relat Metab Disord 2003; **27:** 784-789.

32   Khoo CM, Sairazi S, Taslim S, Gardner D, Wu Y, Lee J, van Dam RM, Shyong Tai E. Ethnicity modifies the relationships of insulin resistance, inflammation, and adiponectin with obesity in a multiethnic Asian population. Diabetes Care 2011; **34:** 1120-1126.

33   Tai ES, Lim SC, Chew SK, Tan BY, Tan CE. Homeostasis model assessment in a population with mixed ethnicity: the 1992 Singapore National Health Survey. Diabetes Res Clin Pract 2000; **49:** 159-168.

34   Kahn CR. Insulin resistance, insulin insensitivity, and insulin unresponsiveness: a necessary distinction. Metabolism 1978; **27:** 1893-1902.

35   Gallagher D, Heymsfield SB, Heo M, Jebb SA, Murgatroyd PR, Sakamoto Y. Healthy percentage body fat ranges: an approach for developing guidelines based on body mass index. Am J Clin Nutr 2000; **72:** 694-701.

36   Gallagher D, Visser M, Sepulveda D, Pierson RN, Harris T, Heymsfield SB. How useful is body mass index for comparison of body fatness across age, sex, and ethnic groups? Am J Epidemiol 1996; **143:** 228-239.

37   Bradley D, Magkos F, Klein S. Effects of bariatric surgery on glucose homeostasis and type 2 diabetes. Gastroenterology 2012; **143:** 897-912.

38   Rattarasarn C, Leelawattana R, Soonthornpun S, Setasuban W, Thamprasit A, Lim A, Chayanunnukul W, Thamkumpee N. Relationships of body fat distribution, insulin sensitivity and

cardiovascular risk factors in lean, healthy non-diabetic Thai men and women. Diabetes Res Clin Pract 2003; **60:** 87-94.

39   Dattilo AM, Kris-Etherton PM. Effects of weight reduction on blood lipids and lipoproteins: a meta-analysis. Am J Clin Nutr 1992; **56:** 320-328.

40   de Leiva A. What are the benefits of moderate weight loss? Exp Clin Endocrinol Diabetes 1998; **106 Suppl 2:** 10-13.

41   Goldstein DJ. Beneficial health effects of modest weight loss. Int J Obes Relat Metab Disord 1992; **16:** 397-415.

42   Kirk E, Reeds DN, Finck BN, Mayurranjan SM, Patterson BW, Klein S. Dietary fat and carbohydrates differentially alter insulin sensitivity during caloric restriction. Gastroenterology 2009; **136:** 1552-1560.

43   Muscelli E, Camastra S, Catalano C, Galvan AQ, Ciociaro D, Baldi S, Ferrannini E. Metabolic and cardiovascular assessment in moderate obesity: effect of weight loss. J Clin Endocrinol Metab 1997; **82:** 2937-2943.

44   Pi-Sunyer FX. Short-term medical benefits and adverse effects of weight loss. Ann Intern Med 1993; **119:** 722-726.

45   Pasanisi F, Contaldo F, de Simone G, Mancini M. Benefits of sustained moderate weight loss in obesity. Nutr Metab Cardiovasc Dis 2001; **11:** 401-406.

46   Escalante-Pulido M, Escalante-Herrera A, Milke-Najar ME, Alpizar-Salazar M. Effects of weight loss on insulin secretion and in vivo insulin sensitivity in obese diabetic and non-diabetic subjects. Diabetes Nutr Metab 2003; **16:** 277-283.

47   Mazzali G, Di Francesco V, Zoico E, Fantin F, Zamboni G, Benati C, Bambara V, Negri M, Bosello O, Zamboni M. Interrelations between fat distribution, muscle lipid content, adipocytokines, and insulin resistance: effect of moderate weight loss in older women. Am J Clin Nutr 2006; **84:** 1193-1199.

48   Klein S, Wadden T, Sugerman HJ. AGA technical review on obesity. Gastroenterology 2002; **123:** 882-932.

49   Jensen MD, Ryan DH, Apovian CM, Ard JD, Comuzzie AG, Donato KA, Hu FB, Hubbard VS, Jakicic JM, Kushner RF, Loria CM, Millen BE, Nonas CA, Pi-Sunyer FX, Stevens J, Stevens VJ, Wadden TA, Wolfe BM, Yanovski SZ. 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society. J Am Coll Cardiol 2014; **63:** 2985-3023.

50   Miller DS, Parsonage S. Resistance to slimming: adaptation or illusion? Lancet 1975; **1:** 773-775.

51   Magkos F, Fraterrigo G, Yoshino J, Luecking C, Kirbach K, Kelly SC, de las Fuentes L, He S, Okunade AL, Patterson BW, Klein S. Effects of moderate and subsequent progressive weight loss on metabolic function and adipose tissue biology in people with obesity. Cell Metabolism in press**:**

52   Petersen KF, Dufour S, Morino K, Yoo PS, Cline GW, Shulman GI. Reversal of muscle insulin resistance by weight reduction in young, lean, insulin-resistant offspring of parents with type 2 diabetes. Proc Natl Acad Sci U S A 2012; **109:** 8236-8240.

53   Pattyn N, Cornelissen VA, Eshghi SR, Vanhees L. The effect of exercise on the cardiovascular risk factors constituting the metabolic syndrome: a meta-analysis of controlled trials. Sports Med 2013; **43:** 121-133.

54   Conn VS, Koopman RJ, Ruppar TM, Phillips LJ, Mehr DR, Hafdahl AR. Insulin sensitivity following exercise interventions: Systematic review and meta-analysis of outcomes among healthy adults. J Prim Care Community Health 2014; **5:** 211-222.

55   Magkos F, Sidossis LS. Exercise and insulin sensitivity. Where do we stand? You'd better run! European Endocrinology 2008; **4:** 22-25.

56   Ross R, Dagnone D, Jones PJ, Smith H, Paddags A, Hudson R, Janssen I. Reduction in obesity and related comorbid conditions after diet-induced weight loss or exercise-induced weight loss in men. A randomized, controlled trial. Ann Intern Med 2000; **133:** 92-103.

57   Weiss EP, Racette SB, Villareal DT, Fontana L, Steger-May K, Schechtman KB, Klein S, Holloszy JO, Washington University School of Medicine CG. Improvements in glucose tolerance and insulin action induced by increasing energy expenditure or decreasing energy intake: a randomized controlled trial. Am J Clin Nutr 2006; **84:** 1033-1042.

58   Catenacci VA, Wyatt HR. The role of physical activity in producing and maintaining weight loss. Nat Clin Pract Endocrinol Metab 2007; **3:** 518-529.

59   Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; **28:** 412-419.

60   Breda E, Cavaghan MK, Toffolo G, Polonsky KS, Cobelli C. Oral glucose tolerance test minimal model indexes of beta-cell function and insulin sensitivity. Diabetes 2001; **50:** 150-158.

61   Breda E, Toffolo G, Polonsky KS, Cobelli C. Insulin release in impaired glucose tolerance: oral minimal model predicts normal sensitivity to glucose but defective response times. Diabetes 2002; **51 Suppl 1:** S227-233.

62   Dalla Man C, Campioni M, Polonsky KS, Basu R, Rizza RA, Toffolo G, Cobelli C. Two-hour seven-sample oral glucose tolerance test and meal protocol: minimal model assessment of beta-cell responsivity and insulin sensitivity in nondiabetic individuals. Diabetes 2005; **54:** 3265-3273.

63   Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol 1949; **109:** 1-9.

64   Bruce RA, Lovejoy FW, Jr., et al. Normal respiratory and circulatory pathways of adaptation in exercise. J Clin Invest 1949; **28:** 1423-1430.

65   Bruce RA, Pearson R, et al. Variability of respiratory and circulatory performance during standardized exercise. J Clin Invest 1949; **28:** 1431-1438.

66   DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979; **237:** E214-223.

67   James WPT, Schofield EC. Human Energy Requirements: A Manual for Planners and Nutritionists. Oxford: Oxford University Press, 1990.

68   Bergman RN, Ader M, Huecking K, Van Citters G. Accurate assessment of beta-cell function: the hyperbolic correction. Diabetes 2002; **51 Suppl 1:** S212-220.

69   Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW, Neifing JL, Ward WK, Beard JC, Palmer JP, et al. Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects. Evidence for a hyperbolic function. Diabetes 1993; **42:** 1663-1672.

70   Mather KJ, Hunt AE, Steinberg HO, Paradisi G, Hook G, Katz A, Quon MJ, Baron AD. Repeatability characteristics of simple indices of insulin resistance: implications for research applications. J Clin Endocrinol Metab 2001; **86:** 5457-5464.

71   Morris AD, Ueda S, Petrie JR, Connell JM, Elliott HL, Donnelly R. The euglycaemic hyperinsulinaemic clamp: an evaluation of current methodology. Clin Exp Pharmacol Physiol 1997; **24:** 513-518.

72   Soop M, Nygren J, Brismar K, Thorell A, Ljungqvist O. The hyperinsulinaemic-euglycaemic glucose clamp: reproducibility and metabolic effects of prolonged insulin infusion in healthy subjects. Clin Sci (Lond) 2000; **98:** 367-374.

73   Succurro E, Marini MA, Frontoni S, Hribal ML, Andreozzi F, Lauro R, Perticone F, Sesti G. Insulin secretion in metabolically obese, but normal weight, and in metabolically healthy but obese individuals. Obesity (Silver Spring) 2008; **16:** 1881-1886.

74   Magkos F, Fabbrini E, Korenblat K, Okunade AL, Patterson BW, Klein S. Reproducibility of glucose, fatty acid and VLDL kinetics and multi-organ insulin sensitivity in obese subjects with non-alcoholic fatty liver disease. Int J Obes (Lond) 2011; **35:** 1233-1240.

75   Tripathy D, Almgren P, Tuomi T, Groop L. Contribution of insulin-stimulated glucose uptake and basal hepatic insulin sensitivity to surrogate measures of insulin sensitivity. Diabetes Care 2004; **27:** 2204-2210.

76   Deurenberg P, Deurenberg-Yap M, Guricci S. Asians are different from Caucasians and from each other in their body mass index/body fat per cent relationship. Obes Rev 2002; **3:** 141-146.

77   Deurenberg-Yap M, Schmidt G, van Staveren WA, Hautvast JG, Deurenberg P. Body fat measurement among Singaporean Chinese, Malays and Indians: a comparative study using a four-compartment model and different two-compartment models. Br J Nutr 2001; **85:** 491-498.