*Study Protocol including Statistical Analysis Plan*

**Official Study Title: Extreme Exercise and Energy Expenditure (4E) Study**

**NCT Number: N/A**

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## BACKGROUND

Although there is robust evidence that a physically active lifestyle including regular leisure time physical activity and exercise (>150 min per week of moderate to vigorous exercise per week) is associated with a reduced mortality risk and improved cardiometabolic health (1), some scientists have promoted the notion (which has certainly been taken up the lay press) that if one wants to lose weight, focus should be placed on caloric restriction rather than on exercise (2, 3). Several academic debates have been held on this issue (4, 5) which have left some health professionals and the public confused and perplexed (6).

Our research group has been active in this area on several fronts. First, we have pioneered the notion that in cardiovascular and “metabolic” medicine, the health risk of overweight and obesity was not appropriately assessed by an index based on body weight (body mass index, which is expressed in kg/m2) (7-9). For instance, using imaging technology (computed tomography), we were among the first, more than 30 years ago, to begin to extensively study regional body fat distribution, showing that excess visceral adipose tissue accumulation was more closely related to an atherogenic and diabetogenic cardiometabolic risk profile than excess body weight or total body fat (7). As sedentary individuals with a diet of poor nutritional value (rich in processed fat and added sugar - promoting preferential visceral fat deposition) are also often characterized by a lower muscle mass and by high levels of abdominal visceral fat (9), several lifestyle intervention studies that we and others (10, 11) have conducted in viscerally obese sedentary men have shown that with regular exercise, one could lose visceral adipose tissue while gaining muscle mass, which would lead to trivial or sometimes no changes in body weight. Thus, if body composition is altered by an energy deficit produced by regular exercise, we have proposed that weight loss may sometime be misleading as an outcome as it may not always reflect favorable changes in body composition (muscle mass increasing) and in visceral adiposity and levels of ectopic fat (decreasing) (12). On that basis, we have proposed that changes in waist circumference could be more useful than body weight loss in the evaluation of the benefits of a lifestyle modification program involving regular endurance exercise such as brisk walking, jogging, or biking (12). For instance, we have previously reported that waist circumference was rather sensitive to lifestyle changes involving regular physical activity/exercise (10). Accordingly, lifestyle intervention studies have shown that a reduction of 4 cm in waist girth was predictive of a 58% reduction in the risk of developing type 2 diabetes among individuals who were initially characterized by abdominal obesity and glucose intolerance (13, 14).

Thus, there is a need to propose a paradigm shift among health professionals to evaluate the impact of lifestyle intervention programs involving physical activity/exercise in high-risk overweight obese individuals with visceral obesity: Weight loss is no longer the optimal or most clinically relevant outcome – losses of visceral adiposity and ectopic fat may be more relevant clinically.

In 2015, a member of our team was asked to write an editorial (15) on a spectacular study which provided very relevant data to the debate about diet vs. exercise and weight loss. In that impressive exercise study, recreational middle-aged cyclists were required to bike 2,700 km over 14 days and asked to try to match their energy intake to their very large energy expenditure, the objective being not to lose weight over the course of the intervention. Thus, the daily exercise “prescription” was huge as the authors wanted to test the ability of these elderly and fit recreational cyclists to match their energy intake to their energy expenditure. The most striking and important finding of this unusual study (16) is that despite the huge energy expenditure associated with the 2,700 km bike expedition, participants did not lose weight. However, such lack of change in body weight was completely misleading in terms of energy balance and changes in body composition. Indeed, the cyclists lost 2.2 kg of body fat while gaining 2.5 kg of fat-free mass, a remarkable finding considering that such changes were observed over a period of only two weeks.

Results of this study provide additional evidence that body weight is a poor outcome to assess the effect of a physical activity/exercise program. In our previously published exercise training studies, a common observation has been the disconnect between changes in body weight and changes in body composition, the latter being sometime observed even in the absence of weight loss (10). As weight poorly tracks changes in body composition induced by exercise, no wonder why exercise is indeed perceived as not being helpful to lose weight by some health professionals. We propose that body weight is a misleading outcome when comes the time to evaluate the clinical benefits of exercise in individuals with overweight/obese (9).

One remaining and clinically important issue remains to be addressed regarding the effects of a large volume of exercise not inducing weight loss: to what extent substantial changes in visceral/ectopic fat could have been observed had imaging data been available? In addition, it would have been interesting for the 2,700 km bike intervention study to report changes in waist circumference, this metric being more sensitive to regular exercise than body weight (12). In our previous intervention studies, it has been our experience to find participants losing quite a bit of visceral fat and dropping their waistline by several centimeters, while not losing any body weight.

Thus, on the basis of the above observations, we put forward the hypotheses that:

1- a very large volume of exercise (1,144 km of bicycle performed for 7 consecutive days) will induce major changes in body composition and in levels of visceral adipose tissue/ectopic fat in a sample of middle-aged recreational cyclists who will be asked to match their energy intake to their very large energy expenditure during the course of the one-week extreme exercise intervention. Thus, in a study where, by design, no weight loss is planned nor wanted, we expect to see major changes in body composition (slight increase in muscle mass and substantial reductions in the size of visceral adipose tissue and ectopic fat depots);

2- changes in levels of visceral adipose tissue and ectopic fat will be better reflected by changes in waist circumference – which should decrease in the absence of body weight loss.

We recognize that this is an extreme exercise prescription that has little relevance to public health. However, results of this study will be very important in shedding light on the importance of shifting our attention from body weight loss to other, more clinically relevant fat depots. Thus, the purpose of the study is to provide evidence, through an extreme exercise prescription, that weight loss is not the appropriate outcome to evaluate the effects of exercise on visceral adiposity and ectopic fat depots (e.g. liver fat and epi/pericardial fat) which will likely be mobilized to a very significant extent in only one week.

**Research Protocol**

A sample of eleven recreational middle-aged male cyclists (aged 50 to 66 years) able to perform such a large volume of exercise over a period of one week and asymptomatic for CVD will be recruited. The cyclists will be relatively fit with previous participation in demanding recreational cycling activities but not retired competitive athletes. From their age, we expect them to have subclinical levels of visceral adipose tissue/ectopic fat (these depots increase in size with age).

For this study, participants will be asked to partake in several evaluations: fasting plasma lipoprotein-lipid profile and inflammation markers, glycated hemoglobin, cardiorespiratory fitness, submaximal exercise test including measurement of energy expenditure, resting and exercise blood pressure and heart rate (HR), evaluation of regional adiposity, liver fat content, epi/pericardial fat, nutritional quality, and level of physical activity. After baseline evaluations, participants will be asked to alternately bike 208 km and 104 km per day on a pre-specified course for seven consecutive days. They will be accompanied during each of the seven bike rides by research professionals in a recreational vehicle. Participants' weight, body composition and waist circumference will be measured under standardized conditions in the morning after an overnight fast and after the exercise. Their HR will be continuously monitored, and participants will wear accelerometers to estimate their daily exercise-related energy expenditure. Foods and fluids will be provided to participants and recorded. Again, from the expected large energy expenditure predicted with HR-EE equations (between 6,000 and 7,000 kcal per day), participants will be asked to eat at least a pre-specified number of calories to be adjusted on the daily basis during the week depending upon subjects evolving body weight recorded every morning in standardized conditions. At the end of the 1,144 km/7-day bike ride, baseline evaluations will be repeated with the exception of the maximal exercise treadmill test, nutritional quality, and level of physical activity. To facilitate the conduct of the protocol, the eleven participants will be evaluated and followed in two distinct groups.

## METHODS

A comprehensive set of body fat distribution/ectopic fat variables will be measured on all participants as well as lipids and apolipoproteins, cardiorespiratory fitness, submaximal exercise test with HR and energy expenditure measured by indirect calorimetry, energy expenditure and energy intake evaluation. Questionnaire on nutritional quality and physical activity level will also be performed.

### *Resting Blood Pressure and Electrocardiogram (ECG)*

Blood pressure measurements will be obtained on all subjects by the same nurse using a sphygmomanometer and a stethoscope as previously described (17). Resting blood pressure will be recorded in a supine position after a 15-minute rest. A resting 12-lead ECG will also be performed.

### *Anthropometry and Body Composition*

Height, body weight and waist circumference (18) will be measured using standardized and previously described procedures. The measurement of body composition will be assessed using a bio-impedance device, the InBody 570 (InBody Co., South Korea), which provides an analysis of the body per compartment (arms, trunk, and legs). This exam is designed to measure body water which in turn allows to measure the lean mass and body fat. Bio-impedance will be performed at pre- and post-evaluations as well as every morning prior to the 7-day bike rides.

### *Fasting Plasma Lipoprotein-Lipid and Apolipoproteins Profiles*

Samples are collected in EDTA tubes in the morning after a 12-hour fast while the participants are in a supine position. Cholesterol and triglyceride levels are determined in plasma using automated techniques (20, 21). Apolipoprotein (apo) A1 and B levels were measured on a Dimension Vista 500 System (Siemens Healthineers, Germany) by luminescent oxygen channeling assay on previously frozen plasma samples (-80°C) according to the manufacturer’s instructions.

### *Abdominal Subcutaneous and Visceral Adipose Tissue*

All imaging will be performed on a Magnetic Resonance Imaging (MRI) 3T system (Ingenia; Philips Healthcare, software version R5.1.9, Netherlands) using the integrated two-channel body coil for excitation and a 28-channel body array surface coil for signal reception. Axial sequence will cover the abdominal region from L2 to S1. Analyses will be performed at the level of L4-L5 intervertebral disc.

### *Measurement of Liver Fat*

Proton Magnetic Resonance Spectroscopy (MRS) will be performed to determine liver fat content (22).

### *Measurement of Epicardial and Pericardial Fat*

Levels of epicardial and pericardial fat are both related to features of the metabolic syndrome (23-25) and visceral adiposity (25). We therefore intend to measure epicardial (covering the epicardium within the pericardium) and pericardial (outside the pericardium) fat depots by MRI (26). Epicardial and pericardial fat is routinely measured in the non-invasive cardiovascular imaging laboratory at the research center.

### *Maximal Exercise Test (ETT)*

To assess cardiorespiratory fitness (maximal oxygen consumption, VO2 max), all subjects will perform the same symptom-limited maximal exercise test on an Excalibur Sport cycle ergometer (Lode B.V., Netherlands) linked to a Cardio2 Ultima gas analyzer system (MGC Diagnostics, Minnesota, MI, USA). The incremental protocol consists of 3-min steps with increases in the workload of 30 or 35 watts per step. Cardiologists blinded as to LV diastolic status will supervise all exercise tests. Blood pressure will be recorded with an automatic sphygmomanometer and HR will be recorded continuously during exercise. Raw data and average 12-lead ECG will monitor cardiac status every 30 seconds during the exercise test as described (27).

### *Submaximal Exercise Test (to determine energy expenditure)*

The energy expenditure will be estimated using the HR-VO2 relationship. VO2 will be estimated by referring to regression lines of HR on VO2 obtained during the measurement of resting metabolic rate and the energy cost of submaximal activities such as sitting, standing, slow walking, fast walking and cycling at various HRs. Measurements of VO2 during these activities will be performed using indirect calorimetry. The calculation of energy expenditure will be made after stabilization of VO2 and CO2 output, usually during the last 2-3 min of each activity as previously described (28).

***Lifestyle Habits Measurements***

Accelerometry (Acti-Graph, Pensacola, FL, USA) recording will be used to average the daily energy expenditure at baseline. Sleep time and numbers of steps will also be provided by the accelerometer. Accelerometer will be used to record 24-hour HR during the 7-day bike ride. HR will be used to extrapolate energy expenditure.

The quality of diet will be evaluated using the validated Dietary Screening Tool (DST) for the assessment of a nutritional quality score which can vary from 0 to 100 (29). Dietary habits will be monitored using a validated quantitative web-based food frequency questionnaire. During the 7-day bike ride, food and liquid intakes will be detailed, weighed, and recorded.

Finally, all the methodology proposed in the present protocol is well established and currently used by the team of investigators. We have all the facilities and equipment to conduct the research project as proposed.

## STATISTICAL ANALYSES

Data will be expressed as mean ± SD. The analysis of the main variables will be the differences in changes in visceral adipose tissue and ectopic fat as well as cardiometabolic risk markers to document changes following the extreme exercise week. A paired t-test will be used with a significance level with a *P* value below 0.05.

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