Effect of exercise on loss of muscle protein and muscle function during 7 days fasting

The prevalence of obesity is high in most Western countries, and obesity is associated with increased risk for a number of diseases like type 2 diabetes, cardiovascular disease, and some types of cancer. Obesity results from overconsumption of energy often in combination with low physical activity, and is associated with high risk for metabolic diseases and type 2 diabetes. Recommended weight reduction and physical activity are part of the treatment for overweight people with metabolic disease. Several strategies are used to reduce body weight, where reduced caloric intake is a cornerstone. Exercise is sometimes included, but to use 500 kcal/day during exercise is not easy.

Many different approaches are used to reduce bodyweight. Periods without food, and even starvation, have been part of the human evolution, and human metabolism allow survival and function in periods without food. Recently several weight loss regimes have included periods of fasting and intermittent fasting has become popular. Examples are the 5-2 diet (Heilbronn et al., 2005). Humans have a resting metabolic rate of ~1,500 kcal/day and activity accounts typically for ~800 kcal/day. The weekly energy requirement corresponds to less than 2 kg fat. The major challenge for the human body is that the brain uses 100-120 g of glucose per day. The liver contains about 100 g of glycogen that can be broken down to supply blood glucose. The liver can also make glucose via gluconeogenesis with amino acids as substrates, but this causes degradation of muscle protein.
A major concern of reducing body weight is that not only fat mass decreases, and the loss of muscles mass is unwanted in particular in older people. Some claim that periods of fasten is beneficial, and there are convincing evidence that energy restriction increases longevity in several animal models (Fontana & Partridge, 2015).

The aim of the present study was to investigate excretion of nitrogen and loss of muscle mass and function during one week of fasting with or without exercise. We will also investigate metabolic adaptations in muscle and adipose tissue during one week fasting with or without exercise. Our hypothesis is that exercise reduces nitrogen excretion and preserves muscle mass, which is beneficial to maintain muscle function. We believe data from the study will be important to make better recommendation about strategies for reduction of body weight.

OUTCOMES

Primary outcomes:

1. Loss of urinary nitrogen
2. Muscle strength and muscle mass.

Secondary outcomes:

1. Plasma concentrations of metabolites.
2. Continuous glucose monitoring.
3. Plasma concentration of hormones and secreted proteins.
4. Maximal oxygen uptake (VO2max).
5. Gene expression in skeletal muscles and adipose tissue.
6. Reaction time and cognitive function and.
7. Microbiota in faeces.

DESIGN AND PARTICIPANTS

Study inclusion criteria

- 18-45 years old
- BMI between 22-30 kg/m^2
- Fat percentage higher than 12% on DEXA measurement
- Some experience with exercise
- Healthy individuals

Study exclusion criteria

- No diseases, such as cardiovascular disease, diabetes and other disease that affect metabolism.
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Participants in the project will perform a number of tests before, during and after 7 days of fasting. An overview of the tests, blood samples, exercise and muscle biopsies is shown schematically in the figure below.

Participant monitoring

Participants will be examined daily during the intervention and contacted by phone during the days to ensure no adverse response, such as electrolyte imbalance, hypoglycemia, ketoacidosis, cardiac arrhythmias (though activity tracker), dehydration, or organ failure. If participants report any related symptoms, fasting will immediately be terminated and participants will be transferred to the hospital for immediate treatment and refeeding. Exclusion of participants with reported diseases will minimize the likelihood of adverse events.

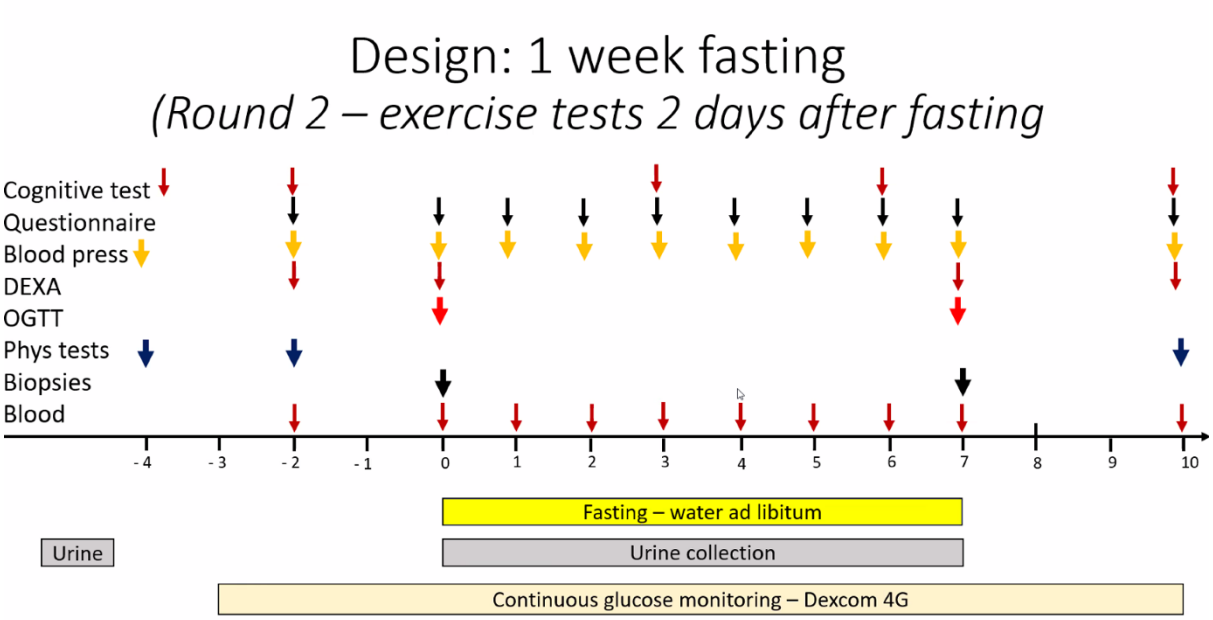

Figure 1: Schematic overview of tests and collection of urine, blood samples and muscle biopsies. It also shows when training should be carried out.

Habituation test:

A familiarisation of the power, strength, lactate profile and VO2max test will be performed eight days prior to the start of the fast. The purpose of running through the tests is to familiarise the subjects with the tests that will be carried out later.

Resting metabolism
Resting metabolic rate refers to how much energy the body consumes at rest. The subject must turn up fasting, preferably in the morning. An oxygen mask is placed over the nose and mouth and resting metabolism is tested by measuring oxygen uptake while lying completely still.

Body composition
Measurement of body composition using a Dual Energy X-ray Absorptiometry (DEXA). The machine can distinguish between bone mass, fat mass and lean body mass. Two measurements will be performed before fasting and two measurements at the end of fasting.

Biopsy
Tissue samples will be taken from the vastus lateralis and subcutaneous fat in the abdominal region before fasting begins and after 3 and 7 days of fasting. In the muscle biopsies, glycogen will be measured as well as transcription, expression and phosphorylation of proteins involved in the metabolism of ketone bodies and other proteins with other functions. In the fat biopsies, the expression of proteins involved in the metabolism of fat will be measured.

Power/power output
A test of maximum power production (watts) over a period of 10 seconds will be carried out on an exercise bike. The subjects will warm up for 10 minutes and perform a maximum sprint of 10 seconds.

Strength
Test of maximum isometric force and isokinetic force in the knee extensors (quadriceps femoris) and hamstrings isometrically and at four different angular velocities in a dynamometer. There will be three warm-up repetitions at lower loads before testing maximum strength and force production at different angular speeds.

Lactate profile test and VO2max
The subject will work for five minutes with a gradually increasing load. For each load, VO2 and lactate are measured for the last two minutes. Heart rate is continuously recorded throughout the lactate profile test. The lactate profile test ends at 4 mmol lactate in the blood. After the lactate profile test, the subjects cycle slowly for ten minutes before performing the staircase test, which is used to measure VO2max. In the staircase test, the intensity is increased every minute until the subject reaches exhaustion.

Blood tests
Blood samples (2x 7ml) are taken every day to measure metabolites (e.g. ketone bodies, fatty acids) and hormones. We will also study peptides that regulate hunger and satiety.

Reaction test
Time to response to visual signal (light or figure on PC) will be used as a test of reaction time.

Questionnaire
A visual analogue scale (VAS) will be used to assess subjects' hunger, fatigue, initiative and cravings for different types of food.

Nitrogen balance
All urine during the fasting period is collected and submitted when you arrive for blood tests. The purpose of collecting urine is to obtain a measure of protein degradation during fasting. In addition to total nitrogen, 3-mithylhistidine and other metabolites should be measured.

Faeces sample
Subjects must provide a faecal sample before and after 7 days of fasting. Intestinal bacteria must be studied by analysing gene transcription. The analyses must be performed by a partner.

Activity tracker
Subjects will wear an activity tracker for a period of 7 days before and throughout Lent. The purpose is to record whether the subjects' activity patterns change during fasting.

Continuous measurement of plasma glucose
Subjects should wear a sensor (Dexcom G4) for continuous measurement of plasma glucose concentration.

Meal test
A liquid, standardised meal will be given and blood samples will be taken before and after 30, 60, 90, 120, 180 and 240 minutes after the meal to measure glucose, fatty acids, amino acids and hormones.

Cognitive function (CANTAB)
Four times during the project period, the subjects will complete a test of cognitive function on an iPad, using a software programme called CANTAB. CANTAB contains a test battery that includes mapping of memory, learning, attention, reaction time, etc. We want to test Reaction Time (RTI), Rapid Visual Information Processing (RVP), Spatial Working Memory (SWM), Paired Associates Learning (PAL), Emotion Recognition Task (ERT). These tests are intended to map cognitive function during the fasting period.

Measurement of blood volume.
You do not need to take this measurement to participate in the study. The haemoglobin mass will be measured through the "CO-rebreathing method" twice before and after fasting. The procedure consists of capillary puncture on a fingertip and two capillary samples. A known amount of carbon monoxide (CO) gas is then rebreathed for 2 minutes before two capillary samples are taken.