

# IceProQualita

## Protocol

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### Intervention period

September 1st 2008, to December 20th 2009.

### Background

Sarcopenia, the loss of muscle mass and strength during aging, is one of the main causes of the functional decline frequently observed among elderly people.<sup>1,2</sup> Therefore, it is considered very important to establish therapies that prevent or delay sarcopenia.

Regular resistance training is an effective way to increase muscle mass and strength in elderly people.<sup>3</sup> It has also been suggested that strategies such as increased protein intake<sup>4</sup> and/or supplementation with essential amino acids, in particular leucine<sup>5</sup>, could maximize gains in muscle mass or strength during a resistance exercise program. In that regard, research has been directed towards whey proteins due to their high quality.<sup>6</sup> Studies in young people indicate that whey protein ingestion directly after exercise stimulates muscle protein synthesis more than other protein sources.<sup>7,8</sup>

The beneficial effect of whey protein is thought to occur primarily due to their very high concentration of essential amino acids, particularly leucine (up to 14%).<sup>9</sup> In general, studies suggest that ingestion of essential amino acids is an effective way to stimulate muscle protein synthesis in

both young<sup>10,11</sup> and elderly individuals,<sup>12</sup> whereas non-essential amino acids appear to have no detectable effect.<sup>12,13</sup> Therefore, it seems reasonable to assume that ingestion of whey protein supplements, with their high amount of essential amino acids, would result in greater gains in muscle mass after a period of resistance exercise in elderly people than, for example, isocaloric carbohydrate supplements. However, studies on the effect of whey protein on muscle mass and strength gain in elderly people are few and it is unclear whether findings from short time experiments on muscle protein synthesis can be extrapolated to a long-term increase in muscle mass or strength.

## Hypothesis

Sarcopenic participants receiving whey protein supplementation or milk during resistance training will experience greater gains in lean body mass, strength, and physical function compared to participants receiving isocaloric carbohydrate.

## Study design

Subjects will participate in a 12-week randomized and controlled resistance exercise intervention program, designed to increase strength and muscle mass of all major muscle groups. All data will be obtained at baseline and again at the end of the study.

## Subjects

It is planned to recruit 250 participants, older than 65 years, and to keep the proportion of men and women similar. Volunteers will be recruited by advertisement posters in Hrafnista in Hafnarfjörður, a home for the elderly in the capital area of Iceland, and other areas in the capital area of Iceland. If cardiovascular disease symptoms will be detected during screening, participants will be encouraged to contact their physician for medical clearance.

## Exclusion criteria

Exclusion criteria are age younger than 65 years, low cognitive function (Mini-Mental State Examination (MMSE) <19 points), evidence of uncontrolled coronary heart disease, major orthopedic disease, and pharmacological interventions with exogenous testosterone or other drugs known to influence muscle mass. Furthermore, all participants have to be free of any musculoskeletal disorders or other disorders that could affect their ability to complete their training and testing.

## Ethical considerations

Applications will be sent to the Icelandic National Bioethics Committee and the Icelandic Data Protection Authority for approval, and the study will follow the Helsinki declaration guidelines. Informed written consent will be obtained from all participants prior to data collection.

## Dietary intervention

Participants will be randomly assigned to one of three different supplement drinks, produced and developed by Mjólkursamsalan (Icelandic dairy producer), which will be packaged into 250 mL brick-style aseptic cartons. The drinks will be based on a sweet whey concentrate (lean cream), low-fat milk protein concentrate, and carbohydrates (placebo).

The supplement drinks will be provided in identical containers and flavoured to mask the contents. All of the drinks will be isocaloric and have the same amount of fat. The whey protein drink and low-

fat milk drink will contain the same amount of protein and carbohydrates, and will only differ from the carbohydrate drink in their protein and carbohydrate contents. Participants will ingest 250 mL of the supplement immediately after each workout, under supervision of study staff, while maintaining their habitual daily diet.

### **Sweet whey concentrate (lean cream)**

The whey protein drink will contain whey in the form of lean cream (LC), which is classified as whey protein concentrate. The whey will be concentrated with ultrafiltration until 60% of the dry matter is protein (WPC-60). The concentrate will then be heat denatured under controlled high shear using a technology based on the APV Shear Agglomerator (ASA). This process gives the whey protein concentrate a cream-like texture and new functional properties. Each 250 ml carton will contain 266 g, which corresponds to 21 grams of protein.

### **Milk drink**

The milk drink (light milk) will consist of normal milk proteins (8%) concentrated by ultrafiltration. Each 250 ml carton will contain 266 g, which corresponds to 21 grams of protein.

### **Carbohydrate drink (placebo)**

A placebo drink containing carbohydrates and no protein will be developed. Taste and color will be similar to the protein drinks.

## **Randomization and masking**

Treatment assignment will be randomized and double blinded. Participants will be randomly allocated to treatment groups following a stratified randomization procedure based on a computer-generated list of random numbers. Randomization will be stratified by gender to make the proportion of men and women in each group equal. The supplement drinks will be provided in identical brick-style cartons and each of the two supplements will have a specific 3-digit-labelling. Investigators and other staff will be kept blind to supplement assignment by the producer of the supplement until the end of the intervention. Furthermore, the supplement drinks will be flavoured to mask the contents.

## **Resistance exercise**

Subjects will exercise for three non-consecutive days per week for 12 weeks in groups of 20-30 individuals. Before the intervention starts, 1-repetition maximum (1-RM) will be assessed using weight machines and by progressively increasing the weight lifted until the participant fails to lift the weight. The first week will be used to teach correct exercise techniques at lower loads (60% of 1-repetition maximum (1-RM)). Thereafter, resistance training will involve 3 sets, where each exercise will be repeated 6-8 times, at 75-80% of 1-RM. The training load will be systematically increased by 5-10% each week in order to keep the number of repetitions per set between six and eight.

Ten different exercises will be performed in weight machines during each training session; seated leg extension, seated leg curl, seated leg press, seated chest fly, seated row, seated pull-down, seated biceps curl, seated triceps curl, seated lower back extension, and seated abdominal curl. Each exercise session will start with a moderate, 10- to 15-minute aerobic warm-up on a treadmill or a stationary exercise bicycle, after which resistance training with weight machines will be performed.

Stretching exercises will be performed at the end of each session. Each session will be supervised by study staff, an athletic trainer, and occasionally a physiotherapist.

## **Dietary intake**

Diet will be assessed using a 3-day weighed food record prior to the study. Participants will weigh and record their food intake for three consecutive days, two weekdays and one weekend day. One of the three days will be a training day. Instructions on how to record the diet will be given orally and in writing. The participants will be provided with electronic scales (PHILIPS HR 2393) and asked to record all food items and drinks. The results of the food records will be typed into an online food calculation program based on the Icelandic food composition database (ISGEM), which contains data on the composition of 1148 foods on the Icelandic market.

## **Anthropometric measurements**

### **Weight**

Body weight (BW) will be measured in light underwear on a digital calibrated scale (model no. 708, Seca, Hamburg, Germany). It will be measured to the nearest 0.1 kg with the subject standing motionless on the scale, with weight equally distributed on each leg, and arms hanging freely.

### **Height**

Height will be measured with a calibrated stadiometer (model no. 206; Seca, Hamburg, Germany) with the subject in a fully erect position against a wall with their weight equally distributed on both feet, and the ankles or knees together. Participants will be asked to position themselves so that the bottom of the eye socket will be approximately level with the opening of the ear canal.

### **Body mass index (BMI)**

BMI will be calculated from the recorded height and weight [ $\text{kg}/\text{m}^2$ ].

### **Waist circumference**

For the measurement of waist circumference, subjects will be asked to stand erect with the abdomen relaxed, arms at the sides, feet together and with their weight equally divided over both legs. The lowest rib margin will be first located. Then the iliac crest will be palpated in the midaxillary line. A flexible tape will then applied horizontally midway between the lowest rib margin and the iliac crest and tied firmly so that it stays in position around the abdomen about the level of the umbilicus. All measures will be performed twice using a tape measure and recorded to the nearest centimeter.

### **Body composition**

Body composition will be assessed by dual energy x-ray absorptiometer (DXA) with Hologic QDR-2000 plus<sup>®</sup>, Hologic Inc., Waltham, MA, USA. The DXA measurements will be conducted at the Icelandic Heart Association, Kopavogur, Iceland. Fat free mass will also be estimated by hand-held bioelectrical impedance analysis (HHBIA) (Body Fat Monitor BF 306, Omron Healthcare UK Ltd, Milton Keynes, UK), CBIA (Bodystat 1500, Bodystat Ltd, Douglas, Isle of Man, British Isles). The two BIA measurements will be conducted at our research unit. Measurements of BIA and DXA will be done within a two-hour interval.

### **Physical strength**

To evaluate muscle strength, grip strength and knee extensor muscle strength (quadriceps strength) will be measured.

### **Quadriceps strength**

Quadriceps strength (maximum voluntary isometric contraction (MVIC)) will be tested with an isokinetic dynamometer (Kin-Com<sup>®</sup> 500H Chattanooga). The subjects will perform three submaximal trials and then perform four MVIC tests for five seconds each, with a 50 second rest between tests. The greatest output will be recorded as the peak torque expressed in Newtons (N).

### **Grip strength**

Grip strength will be measured with a hydraulic hand dynamometer (Baseline<sup>®</sup> Baseline Evaluations Corporation) and the maximal grip strength of three trials will be registered as the subject's grip force in pounds (lbs).

### **Physical function**

Subjects will undergo pre- and post-intervention testing of physical function, including the timed up and go (TUG) test in seconds and a six minute walk for distance (6MW) in meters.

#### **Timed Up and Go test (TUG)**

During the TUG test the subject will be instructed to rise from a chair with a seat height of 43 cm, walk 3 m, turn around, return and sit down again, while wearing ordinary footwear and using customary walking aids if necessary.

#### **Six minute walk for distance (6MWD)**

The 6MWD will be performed indoors, in a spacious gym hall, along a flat, straight line with a hard surface, and conducted according to the guidelines from the American Thoracic Society.<sup>14</sup>

### **Blood pressure**

Baseline fasting blood pressure will be measured in the morning with a Medisana upper arm blood pressure monitor (Medisana AG, Neuss, Germany) after a 12 hour fasting. All blood pressure measurements will be performed by qualified health care staff while the participant sits still and relaxed in sitting position with the upper right arm exposed. During the measurements the subject will be asked not to engage in conversation and the arm of the subject will be allowed to rest on a desk so that the antecubital fossa will be at approximately the same level as the heart. Two readings will be taken at intervals of 2 minutes and the average of those readings will represent the patient's blood pressure. The third reading will be performed when the difference between the first two readings is greater than 10 mmHg. Participants will be asked not to take any antihypertensive drugs for 12 hours prior to the measurements.

### **Biochemical measurements**

Participants will be instructed to avoid strenuous exercise and alcohol consumption the day before the drawing of fasting blood samples at baseline and endpoint. The blood samples will be centrifuged and the serum stored at -80°C for subsequent analyses at the laboratory of the National University Hospital in Reykjavik, Iceland. Blood tests will give information about general blood status. The following blood variables will also be measured.

#### **Hb1aC**

HbA<sub>1c</sub> will be measured using a chromatographic–spectrophotometric assay.

### **Glucose**

Glucose will be analyzed using an enzymatic colorimetric assay and an automated analyzer (Hitachi 911; Roche Diagnostics).

### **Insulin**

Insulin was measured with electrochemiluminescence immunoassay on a Modular Analytics E170 system from Roche Diagnostics (Manheim, Germany).

### **IGF-1**

IGF-1 will be analyzed with a solid-phase, enzyme-labeled chemiluminescent immunometric assay on a Immulite 1000 system from Siemens Healthcare Diagnostics.

### **Triglycerides**

Plasma triglycerides will be analyzed using an enzymatic colorimetric assay and an automated analyzer (Hitachi 911; Roche Diagnostics).

### **High-density lipoprotein (HDL)**

HDL will be determined using polyethylene glycol-modified enzymes and dextran sulfate.

### **Total cholesterol**

Total cholesterol will be analyzed using an enzymatic colorimetric assay and an automated analyzer (Hitachi 911; Roche Diagnostics).

### **Creatinine**

Serum creatinine ( $\mu\text{mol/L}$ ) will be measured by standard dry chemistry methodology on Vitros 750 XRC 700 analyzer (Johnson & Johnson).

### **Health related quality of life**

The HRQL questionnaire that will be used has 12 domains that cover a range of psychological and physical functions; functional status, vitality, social function, physical pain, emotions, general health and mental health. The domains will be summarized into a single general score; quality of life. Each sub-scale in the instrument is calculated for men and women in different age groups and will be converted to a T-score (T-score norm is  $50 \pm 10$ ). The HRQL questionnaire was developed based on several questionnaires that were intended to measure health related quality of life. An Icelandic translation of these questionnaires was presented to 147 individuals, following a detailed cluster analyses of the answers it was decided to develop the instrument,<sup>16</sup> later two questions concerning memory and concentration were added later. This new version of the questionnaire has been tested for the Icelandic population, and used to assess HRQL.<sup>17</sup>

### **Mini mental state examination**

Cognitive function will be assessed with a mini-mental state examination (MMSE) or Folstein test.

### **Demographics and other relevant variables**

All participants will be asked to answer questions regarding their level of education, leisure-time physical activity, professional activity and spouse's profession (before retirement), as well as alcohol and smoking habits. Smoking and alcohol consumption will be categorized as never, former, or current. Information about health problems and medications will also be collected.

### Leisure time physical activity

Leisure time physical activity will be assessed by self-reports and by a yes/no question; “Do you engage in regular physical activity?” It will be shown either as min/day or calculated as kcal/week by multiplying the appropriate kcal score (based on metabolic equivalent of the task values) for each of the activities by the amount of time spent during the week doing the activity.<sup>18</sup> Total physical activity will be defined as the sum of the activity reported on the questionnaire for non-exercise walking (e.g., walking the dog), walking for exercise, and other moderate and vigorous exercise activities.

### Statistical analyses

The data will be entered into the SPSS statistical package, version 20.0 (SPSS, Chicago, IL, USA). Data will be described as mean  $\pm$  standard deviation (SD). Statistical significance will be set at P-value < 0.05. General linear models will be used to detect differences between groups.

### Power calculations

According to power calculations, a sample size of at least 63 participants in each group was required to detect a difference of 1 kg in lean body mass between groups as significant (SD = 2 kg, power = 0.8, P = 0.05).

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