

Rate of Recovery from Strenuous Exercise in Physically Active Older Adults

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Study Protocol

Subjects. A total of 39 healthy men and women were studied. They were divided into three groups according to their age and physical activity status; the young sedentary group, the young exercise-trained group, and the older exercise-trained group. Young and older groups were 19-39 and 50-77 years of age, respectively. Subjects in the trained groups had been performing regular aerobic exercise (primarily running) at least twice or more a week for at least two years. The subjects in the sedentary group had not participated in a regular exercise program for at least one year. Older sedentary subjects were not included in the present study due to safety concerns associated with downhill running. All subjects were nonsmokers. Subjects were free of overt chronic diseases as assessed using medical history. All subjects were free of any physical limitations. All older women were postmenopausal. Three women in the young groups were using oral contraceptives, and other premenopausal women had been experiencing regular menstrual cycles. In premenopausal women who did not use oral contraceptives, all measurements were conducted in early follicular phase of their menstrual cycle because the phase contains the lowest estrogen levels. All subjects gave their written informed consent prior to study participation, and the ethics committee of the University of Texas at Austin approved all procedures.

Procedures. Subjects visited the laboratory on five separate occasions. On the first visit, subjects filled out the standard health research questionnaire. They then underwent a familiarization/measurement session. The familiarization portion was used to introduce the subjects to downhill running on treadmill. Then each subject's maximal oxygen consumption ($\dot{V}O_{2max}$) were measured using a maximum exercise test. On the

second visit, the subjects underwent the baseline measurements, including anthropometry, body composition, markers of muscle damage (plasma concentrations of creatine kinase and myoglobin, visual analog scale of pain and soreness, and joint range of motion) and muscular strength of their leg (maximum isometric contraction). Following the measurements, subjects performed downhill running. After the downhill running protocol, various markers of muscle damage and muscular strength were obtained 24 hours post (the third visit), 48 hours post (the fourth visit) and 72 hours post (the fifth visit). There were at least 3 days between the first and second visits, with no more than 7 days between the two visits. Subjects were instructed to fast at least 3 hours and to avoid any strenuous exercise for the 48 hours before the measurements. Subjects were asked to keep their regular diet throughout the testing sessions.

Maximal oxygen consumption. Maximal oxygen consumption ($\dot{V}O_{2max}$) was measured with an incremental treadmill protocol test as previously described [32]. After a 5-min warm-up period, the subject ran at a speed that corresponded to 70–80% of age-predicted maximal heart rate [31]. Treadmill speed remained constant throughout the test, while the grade was increased by 2% every 2 minutes until volitional exhaustion. A mouthpiece and heart rate monitor (Polar Electro Inc, Lake Success, NY) were worn to collect expired gas and obtain heart rate. The rating of perceived exertion (RPE) scale was obtained every minute. A metabolic cart was used to measure flow and gas composition from expired air collected by the mouthpiece. At least two of the following three criteria were met by each subject: 1) a plateau in oxygen uptake with increasing exercise intensity, 2) a respiratory exchange ratio ≥ 1.1 , and 3) achievement of age-predicted maximal heart rate [31]. $\dot{V}O_{2max}$ was determined by the highest values

of oxygen uptake that were sampled every 20 s. The $\dot{V}O_{2max}$ values were used to set the speed of treadmill during the downhill running.

Downhill running. Subjects performed downhill running at -16% of slope at a speed that corresponded with 65% of their $\dot{V}O_{2max}$ [26]. Subjects warmed up on treadmill on a level grade at the same speed as downhill running for 5 min. Following the warming up period, subjects began downhill running. The downhill protocol consisted of three 15-minute sessions with 5-minute rest intervals in seated position. Heart rate (using Polar heart rate monitor), and RPE (for cardiorespiratory and lower limbs, separately) were recorded every 5 minutes. Similar protocols have been successfully utilized to induce DOMS in young and older adults [7,11,27].

Anthropometric assessment. Height (± 0.1 cm) and body weight (± 0.1 kg) were measured using a physician's balance scale. Body composition was assessed using the 7-site skinfold thickness technique [19,20].

Joint range of motions. A goniometer was used to assess the range of motions of knee (extension) and hip (extension and flexion) joints as indices of muscle stiffness. Each measurement was repeated twice and averaged for both left and right sides. The sites of goniometer were marked with a semi-permanent-ink pen to ensure good day-to-day repeatability. Hip flexion was tested while the subject was placed in the supine position with their knees bent over the edge of the treatment table. The subject was instructed to flex the knee to the chest, and hip flexion angle was assessed. The stationary arm of the goniometer was placed along the lateral midline of the pelvis; the axis of movement placed at the lateral aspect of hip aligned with the greater trochanter, and the moving arm was placed along the lateral midline of the femoral lateral condyle.

Hip extension was assessed while the subject was placed in the prone position. While the pelvis is stabilized, the subject raised their leg off the table. The stationary arm of the goniometer was aligned the lateral midline of the pelvis; the axis of movement was placed on the lateral aspect of the hip aligned with the greater trochanter and the moving arm placed along the lateral midline of the femur aligned with the femoral lateral condyle.

Knee extension was measured with the subject laying supine, with both knee and hip flexed. The opposite leg lied flat on the table throughout the examination. The subject actively extended the knee through the full range of motion until resistance was felt, while the hip was maintained at 90° flexion. The stationary arm of the goniometer was aligned along the femoral greater trochanter; the axis of movement placed at the lateral femoral condyle at the knee joint and the moving arm aligned with the lateral malleolus.

Visual analog pain scale. A validated visual pain scale (VAS) was used to determine the level of muscle soreness [9]. The scale was on a 10-point scale (0 being absence of soreness, 10 being worst imaginable soreness). Subjects were asked to rate the VAS at their quadriceps, calves, and glutes during walking in daily life. In addition, VAS of subject's legs was obtained when angles of hip flexion (VAS-hip flexion), hip extension (VAS-hip extension), and knee extension (VAS-knee extension) were measured.

Muscular strength. Maximum voluntary isometric forces of the dominant knee extensors were measured using the isokinetic dynamometry system (Cybex II, Cybex, Inc, Ronkonkoma, NY). Subjects were seated on the machine, fixed by belts for waist,

shoulders, and thigh. Maximum isometric muscular strength was measured by determining peak torque during knee extension contractions at 90° and 45° of knee angles. Subjects performed three maximum knee extension contraction each for knee angles with 60-second rests between trials. The average peak torque of the three trials was used for further analyses.

Blood samples. Blood samples (5 ml) were drawn from the antecubital vein by a certified phlebotomist. Plasma was separated by centrifugation for 15 minutes. All plasma samples stored at -80°C until biochemical analyses at a later date. Creatine kinase activity and myoglobin concentration, as indices of muscle damage, were analyzed using commercially available ELISA assay kits. Due to the budget constraints, these measurements were measured only in the older trained and young trained groups.

Statistical analyses

Changes in dependent variables of muscular damages over time were compared among three groups by a two-way ANOVA with repeated measures (time x groups). Scheffe's post-hoc analyses were performed when significance was achieved. P-values less than 0.05 were considered significant. Numeric data are expressed as means±SEM. All statistical analyses were performed using StatView (5.0; SAS Institute, Tokyo, Japan) software.