Video Article

Improving Strength, Power, Muscle Aerobic Capacity, and Glucose Tolerance through Short-term Progressive Strength Training Among Elderly People

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Abstract

This protocol describes the simultaneous use of a broad span of methods to examine muscle aerobic capacity, glucose tolerance, strength, and power in elderly people performing short-term resistance training (RET). Supervised progressive resistance training for 1 h three times a week over 8 weeks was performed by RET participants (71±1 years, range 65-80). Compared to a control group without training, the RET showed improvements on the measures used to indicate strength, power, glucose tolerance, and several parameters of muscle aerobic capacity. Strength training was performed in a gym with only robust fitness equipment. An isokinetic dynamometer for knee extensor strength permitted the measurement of concentric, eccentric, and static strength, which increased for the RET group (8-12% post- versus pre-test). The power (rate of force development, RFD) at the initial 0-30 ms also showed an increase for the RET group (52%). A glucose tolerance test with frequent blood glucose measurements showed improvements only for the RET group in terms of blood glucose values after 2 h (14%) and the area under the curve (21%). The blood lipid profile also improved (8%). From muscle biopsy samples prepared using histochemistry, the amount of fiber type IIa increased, and a trend towards a decrease in IIx in the RET group reflected a change to a more oxidative profile in terms of fiber composition. Western blot (to determine the protein content related to the signaling for muscle protein synthesis) showed a rise of 69% in both Akt and mTOR in the RET group; this also showed an increase in mitochondrial proteins for OXPHOS complex II and citrate synthase (both ~30%) and for complex IV (90%), in only the RET group. We demonstrate that this type of progressive resistance training offers various improvements (*e.g.,* strength, power, aerobic capacity, glucose tolerance, and plasma lipid profile).

Video Link

The video component of this article can be found at https://www.jove.com/video/55518/

Introduction

Aging is associated with a loss of muscle mass (sarcopenia), strength, and power. Reduced strength, and probably even more importantly, power, results in immobility, an increased risk of injury, and a reduced quality of life. Resistance training is a well-known strategy to counteract sarcopenia and deteriorating muscle function. A rough estimate of muscle strength can be obtained from the load or number of achieved repetitions. However, this study obtained more detailed and accurate information on muscle function using an isokinetic dynamometer to gather information on the torque during isometric, concentric and eccentric contraction, as well as on the kinetics of force development.

Aerobic capacity, both at the whole-body level (VO_{2max}) and in skeletal muscle, is reduced in elderly people. The decline in heart rate with age explains a large part of the decrease in VO_{2max}[1], but reduced muscle oxidative capacity, largely related to reduced physical activity[2], does contribute. Impaired mitochondrial function may also be involved in the development of sarcopenia and insulin resistance[3]. The muscle aerobic capacity was assessed in muscle biopsies through biochemical analyses of the contents of mitochondrial enzymes and protein complexes located both in the matrix (*i.e.,* citrate synthase) and the inner mitochondrial membrane. In addition, histochemical techniques were used to measure the effect of resistance training on muscle morphology (*i.e.,* fiber type composition, fiber cross-sectional area, and capillary density). An alternative method to assess muscle aerobic capacity would be to use magnetic resonance spectroscopy to measure the rate of creatine phosphate resynthesis after exercise-induced depletion[4]. This method provides an estimate of the *in vivo* muscle aerobic capacity but cannot discriminate between mitochondrial dysfunction and circulatory disorders. Furthermore, the high costs of equipment limit the use of this technique in most laboratories. Aerobic capacity (VO_{2max} and mitochondrial density) can be improved by endurance exercise in both young and old people[5,6]. However, the effect of resistance training on these parameters has been less investigated, especially in elderly subjects, and the results are conflicting[7,8,9,10].

Type 2 diabetes is a widespread disease in the elderly population. Physical inactivity and obesity are major lifestyle-related factors explaining the increased incidence of type 2 diabetes. Low-intensity aerobic exercise is often recommended to subjects with reduced glucose tolerance.

However, it is unclear how strength training in the elderly affects glucose tolerance/insulin sensitivity[11,12]. The most accurate way to measure insulin sensitivity is to use the glucose clamp technique, where the blood glucose is maintained constant by glucose infusion during conditions of elevated insulin[13]. The disadvantages with this technique are that it is time consuming and invasive (arterial catheterization) and requires special laboratory facilities. In this study, the oral glucose tolerance test, which is common in healthcare units, was used. This method is suitable when several subjects are to be investigated for a limited period of time.

The testing and timeline of the experimental procedure can be summarized as follows. Use three separate days for testing before and after an eight-week period, with the same arrangement and approximate time schedules (≥24 h between each day, **Figure 1**). On the first test day, measure: anthropometric data, such as height, body mass, fat-free mass (FFM), and upper leg circumference (*i.e.,* 15 cm above the apex patellae in a relaxed supine position); submaximal cycling ability; and knee muscle strength, as described in steps 4 and 5. Take a muscle biopsy from the thigh on the second test day. For further descriptions, see step 6.1. Test oral glucose tolerance (OGTT) on the last testing day. For further descriptions, see step 7.1. Ask all participants to avoid vigorous physical activity for 24 h and to fast overnight prior to each test day. However, ask them to avoid strenuous physical activity for 48 h before the OGTT test day. Ask them to follow their normal everyday physical activity and diet habits. Note that pre- and post-intervention, both groups' self-reported food intake and type of foods were unchanged.

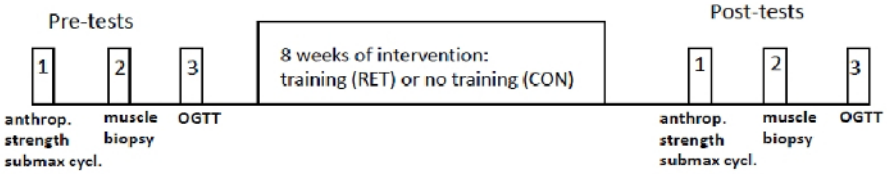

Figure 1: Experimental protocol. Schematic diagram. The timing between the three pre- and post-tests was similar for each subject and was at least 24 h. Further details are given in the text. This figure has been modified from Frank *et al. Scand. J. Med. Sci. Sports*. 2016: 26, 764-73.[28] Please click here to view a larger version of this figure.

This study sought to investigate the effect of short-term resistance training in elderly people on muscle oxidative capacity and glucose tolerance. The second aim was to examine the effect on strength, power, and muscle qualitative improvements (*i.e.,* proteins involved in cell signaling and muscle fiber type composition).

Protocol

The Regional Ethics Committee of Stockholm, Sweden, approved the design of the investigation.

1. Material

1. Recruit relatively healthy women and men 65-80 years old who have BMI values between 20 and 30 $kg \cdot m^{-2}$. Randomize them into two groups. Ensure that the individuals in both groups have relatively low physical activity levels (*i.e.,* moderate daily physical activity and no regular exercise training).
2. Exclude beta-blocker users and those with coronary artery disease and severe neurological or joint problems.
3. Ask the subjects for their written consent after informing them of possible discomfort and risks in the test and training sessions.
4. Balance the resistance training (RET) and control without training (CON) groups in terms of age, sex, and BMI. Ask one group to perform RET under a trainer for 1 h three times a week for eight weeks; the other group will serve as controls (CON).

2. Testing and Training

Note: The eight exercises are standard strength training exercises: seated leg press, seated abdominal crunch, supine chest press, seated back extension, seated shoulder press, seated rowing, seated leg extension (knee extension), and prone leg curl (knee flexion); see **Figure 8** in the Representative Results section.

1. During the first training session, assess the maximal strength at one maximal repetition (1 RM) for each training exercise.
   NOTE: The 1 RM model is commonly used and is defined as the load at which the subject can lift or push the resistance only once but not twice.
   1. Before the start, ask the participant to perform a short warm-up (with a few initial trials at very low weight loads) of the tested exercise. Subsequently increase the load until just below the probable 1 RM value (most often the maximum of 3-4 increased loads). Register the maximal load that the subject can perform only once (= 1 RM).
   2. Measure 1 RM in the eight standard strength training exercises (see **Figure 8** in the Representative Results section). Ask the subjects to rest for at least 2-3 min between each tested exercise.
      NOTE: Strength training equipment was used for all training exercises, including the tests of each training exercise.
2. Ask the whole RET group to perform 1 h of supervised strength training three times a week for eight weeks. Ask the participants to perform, after warm-up, the eight abovementioned standard training exercises. They should repeat an exercise 12 times in each set and perform three sets of each exercise. Allow rest for 1 min between each set and 2-3 min between each exercise.
   1. Ask subjects to perform each exercise as fast as possible during the concentric phase (*i.e.,* muscle shortening phase) and slowly during the eccentric phase (*i.e.,* muscle elongation phase).
      NOTE: Subjects can do the exercises in any order. However, ask them to start and end with a leg exercise and also to try to perform the eight exercises in the presented order. Use strength training equipment for all eight exercises.

2. During each training session, ask the participants to perform three sets at 75-80% of 1 RM for each exercise. Increase the load by approximately 5% the session after when a participant can do 12 repetitions in all three sets of an exercise.

3. Submaximal Cycling Test

Note: Perform the submaximal cycling test on test day 1 (see the Introduction and **Figure 1**).

1. Perform a cycle ergometer test, including two submaximal levels, each for 4 min[14,15]. Set the first work rate to be low (30 W) and the second at 60-120 W, with no pause between the loads on the cycle ergometer.
   NOTE: The first load is the same for all subjects, but the second and last submaximal level should be approximately 65-85% of maximal heart rate for each subject. Both loads should be the same before and after the intervention period of 8 weeks of training.
   1. Base the second-highest load level on familiarization tests done before the trials by asking how physically active the person is and by having the subject initially cycle for a short while; the test leader will form an opinion based on the subject's heart rate as to what final submaximal load is appropriate.
   2. Record the mean steady heart rate (HR) using a heart rate monitor via a chest belt during the last minute on the low and high work rates, by taking the mean of the observed HR at 3:15, 3:30, 3:45, and 4:00 min at each work rate.
   3. Use an ergo-spirometric device to ascertain the composition of gas (O_2 and CO_2) in the expired and inspired air. Register the respiration exchange ratio (RER; *i.e.,* CO_2/O_2), and quantify the RER mean values during the last minute (from four measures every 15 s) at both work rate loads.

4. Knee Extensor Strength: Static, Eccentric, and Concentric Peak Torque and the Rate of Force Development

Note: Perform knee strength measurements on test day 1 (see the Introduction and **Figure 1**).

1. Before the recordings, ask the subject to perform a warm-up by cycling for 8-10 min on a cycle ergometer at submaximal level (i.e., approximately 65-85% of maximal heart rate).
2. Ask the subject to sit on the bench of an isokinetic dynamometer. Fix the subject's trunk with straps over the shoulders and hips. Securely strap the subject's shank to the dynamometer shaft with two straps: one below the knee and one just above the ankle. Align the knee joint axis with the rotational center of the dynamometer shaft.
3. When the subject is secured, assess the maximal voluntary knee strength as the peak torque, with the subject sitting in the isokinetic dynamometer. Initially allow the subject to perform several trials for familiarization with the knee strength equipment (isokinetic dynamometer).
4. Ask the individual to perform four maximal voluntary eccentric and concentric knee extensions (alternately), with the right leg at a constant angular velocity of 30 deg/s. Set the range of motion between 90° and 15° (straight leg = 0°).
   1. In the eccentric task, ask the subject to resist the dynamometer shaft with maximal effort through the whole movement from the 15° to 90° knee angle. In the concentric task, ask the subject to press the lower leg in the dynamometer shaft in a knee extension, as hard as possible throughout the whole motion range.
5. Allow a 4-min rest after the dynamic recordings. Thereafter, assess the static maximal voluntary contraction torque (MVC) four times at a 65° knee angle. In every static trial, ask the subjects, sitting in the same dynamometer, to kick as fast and hard as they can against the dynamometer shaft, which now is fixed (at 65°) and cannot be moved.
6. For torque (strength) signals, convert the analog torque signals to digital using an analog-to-digital converter box connected to the isokinetic dynamometer.
   NOTE: The converter automatically changes the analog signals from the dynamometer to digital signals, which thereafter are automatically exported to the computer where the data are collected.
   1. Set the sampling frequency at 5 kHz in the software analysis program of the computer. Store the digital signals on the computer for a subsequent strength value analysis with the software analysis program.
7. In the subsequent analysis, use the highest value obtained from four trials for each subject in the eccentric, concentric and static measurements. In the software program, click on the highest value of the four trials and write down the strength value shown on the computer screen.
   1. Register the highest peak torque in the eccentric and in the concentric recordings for each subject and the highest strength value among the four static trials.
      NOTE: Isokinetic dynamometer testing of knee extensor strength in a seated position has proper reliability and validity[16,17].
8. Measure the rate of force (torque) development (RFD) during 0-30 ms and 0-200 ms in the highest value found among the static trials. Set the value of zero at the 7.5-Nm level for the onset of contraction for knee extensor strength (time: 0 ms)[18,19]. Move the cursor (in the software program for muscle strength analysis) to the "7.5 Nm" value on the y-scale to obtain the position for 0 ms.
   1. For the pre-test assessment, set the cursor on the 30-ms value (after the time 0 ms). Write down the value showing the raise in Nm at 30 ms (*i.e.,* the increase in Nm from 7.5 Nm = 0 ms). Do the same procedure for the post-test value.
   2. Calculate the increase in percentage for the post-test Nm value (numerator) compared to the pre-test Nm value (denominator) over the period of 0-30 ms. Thus, present the RFD raise in percent from the pre-test to the post-test. Do the same analyses for the time interval of 0-200 ms.

5. Muscle Biopsy

Note: Perform a muscle biopsy on test day 2 (see the Introduction and **Figure 1**).

1. Take a muscle biopsy from the middle portion of the thigh muscle *vastus lateralis* using a conchotome[20].
    1. Prior to the biopsy, inject 1-2 mL of local anesthesia subcutaneously and into the fascia. After a couple of minutes, make an incision with a small scalpel through the skin and fascia, approximately 1/3 of the distance from the patella to the anterior superior iliac spine. Extract about 100-150 mg of muscle tissue using the conchotome.

2. Freeze samples for histochemistry in isopentane cooled to its freezing point in liquid nitrogen and store it at -80 °C. Store a sample of 30-50 mg of muscle tissue.
3. Rapidly freeze the samples for protein analysis in liquid nitrogen and store them at -80 °C. Store a sample of 30-50 mg of muscle tissue.

6. OGTT

Note: Perform OGTT (oral glucose tolerance test) on test day 3 (see the Introduction and **Figure 1**). The time between the exercise and OGTT must exceed 48 h and should be similar between the pre- and post-tests. A 2-h oral OGTT is used to investigate whether frequent blood samples during this time show normal or increased levels, indicating diabetes or prediabetes conditions.

1. Perform the OGTT test in the morning on subjects who have fasted overnight and have not done any strenuous exercise on the testing day or the day before.
2. Take blood samples (4 mL) from supine participants via a venous cannula in the antecubital vein 15 min before and just prior to the intake of glucose, followed by 15, 30, 60, 90, and 120 min after the ingestion of the glucose (75 g of glucose in a 250 g/L solution).
3. Centrifuge the blood samples at 1,500 x g and 4 °C for 10 min and store the plasma at -20 °C for future analysis. Use the samples to perform standard glucose level tests (step 7).
4. For glucose, insulin, and c-peptide, calculate the area under the curve (AUC) by determining the time integral of glucose above basal glucose levels. Use the OGTT results to calculate insulin sensitivity for the whole body using the Matsuda method[21], as per the equation: $10\,000*\sqrt{[(Glucose_{basal}*Insulin_{basal})*(Glucose_{mean}*Insulin_{mean}]}$.

7. Blood Sample Analysis

1. Quantify the glucose concentration in the venous plasma with an automated analyzer. Set the impaired glucose tolerance level at blood glucose values >7.8 mmol/L after a 2-h OGTT[22].
2. Use ELISA kits[22] to perform a plasma analyses of insulin and c-peptide. Use a plate reader. Put the ELISA plates for both insulin and c-peptide in a plate reader (each on a separate occasion).
   NOTE: The plate reader measures the amount of insulin and the amount of c-peptide by measuring the samples on the plate at certain absorbances. Blood lipids TG, HDL, apolipoprotein A1, and apolipoprotein B were analyzed with standard methods at the Karolinska University Hospital, Stockholm, Sweden.

8. Analysis of Muscle Samples

1. Immunoblotting
    1. First, freeze-dry the muscle sample in a lyophilizer at a pressure below 10^{-1} mbar for 12 h. Dissect it so that it is free of blood and connective tissue using a needle and forceps under a light microscope. Store it at -80 °C.
       NOTE: A suitable amount of muscle is between 1 and 5 mg of dry weight, but the protocol can be adjusted to less than 1 mg, all the way to single fibers. Due to the low amount of muscle tissue present in one biopsy, values from that RET participant were not used for immunoblotting.
    2. Homogenize the muscle samples with a mini bead beater in ice-cold buffer (80 µL/mg) composed of 2 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 1 mM ethylenediaminetetraacetic acid (EDTA), 5 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), 10 mM $MgCl_2$, 50 mM ß-glycerophosphate, 1% TritonX-100, 1 mM Na_3VO_4, 2 mM dithiothreitol, 20 µg/mL leupeptin, 50 µg/mL aprotinin, 1% phosphatase inhibitor cocktail, and 40 µg/µL PMSF (phenylmethylsulfonyl fluoride).
        1. Place a scoop of 0.5-mm zirconium oxide beads in each tube with the muscle. Add buffer and homogenize for 2 x 1 min at speed step 7-8 (here, the maximum is 10) and 4 °C.

    3. Centrifuge the homogenate for 10 min at 10,000 x g. Transfer the remaining supernatant to new tubes and discard the pellet containing the structural proteins.
    4. Spectrophotometrically determine the protein concentration in the supernatant with a commercially available kit using a plate reader at 660 nm[23].
        1. Subsequently dilute the samples with 2x Laemmli sample buffer and homogenizing buffer (1:1) to a final protein concentration of 1.5 µg/µL. Heat them to 95 °C for 5 min to denature the proteins. Store the diluted samples at -20 °C prior to analysis.

    5. For Native-polyacrylamide gel electrophoresis (PAGE), load 30 µg of protein from each sample into 18-well precast gradient gels (4-20% acrylamide) and perform electrophoresis at 300 V for 30 min on ice.
    6. Equilibrate the gel in transfer buffer (25 mM Tris base, 192 mM glycine, and 10% methanol) for 30 min at 4 °C. Transfer proteins to polyvinylidene fluoride membranes with 0.2-µm pore sizes at a constant current of 300 mA for 3 h at 4 °C.
    7. To confirm equal loading and transfer, stain the membranes with a total protein stain[24]. For each target protein, load all samples from each subject onto the same gel and run all gels at the same time.

8. Block the membrane for 1 h at room temperature in Tris-buffered saline (20 mM Tris-base, 192 mM NaCl; TBS; pH 7.6) containing 5% non-fat milk.
9. Incubate the membranes over night with primary antibodies (see the Materials List) diluted in TBS containing 2.5% non-fat milk and supplemented with 0.1% Tween-20 (TBS-TM).
10. Following primary antibody incubation, wash the membranes (2 x 1 min plus 3 x 5 min) with TBS-TM and incubate with secondary antibodies (see the Materials List) conjugated with horseradish peroxidase for 1 h at room temperature. Wash again with TBS-TM (2 x 1 min and 3 x 10 min) and again subject them to four additional 5-min washes with TBS.
11. Apply 6-12 mL of chemiluminescent substrate to the membrane for 5 min. Place the membrane between two transparent plastic sheets. Place the membranes in front of a CCD camera blocking external light. Take serial exposures using a chemiluminescent camera filter.
    1. Use the software program to acquire 10 exposures for 2 min, or until the signals are saturated. Use a standard setup, both for the optical filter settings to acquire chemiluminescence, as well as for the lens settings.
12. Use the highest exposure that does not lead to saturation and mark the contours of the band. Quantify the bands as the intensity x mm^2 using the same software. Subtract the background noise from the band intensity. Present the results relative to the total protein stain and express it as the percent change as compared to baseline.

2. Histochemistry
NOTE: The histochemistry technique below is based on methods described in an earlier publication[25].
    1. For histochemistry, cut serial cross sections (10 µm) at -20 °C using a cryostat. Mount the cross-sections on glass slides stored in a glass cuvette and air dry the biopsy slices at room temperature.
    2. Prepare buffer solutions for each pH level for pre-incubation at pH 4.3, 4.6, and 10.3 for ATPase staining[26]. To visualize capillaries, stain the cross-sections using the amylase-PAS method[27].
    3. Calibrate a pH-meter by pouring calibration solutions into labeled calibration beakers. Push the appropriate button to select the pH from the main menu.
        1. Rinse the probe with deionized water and place the probe in the first calibration beaker. Make sure that there are no air bubbles in the membrane. Measure the first calibration solution and then present the next calibration solution (the display will ask for the next solution).
        2. Rinse the probe with deionized water and then place it in the second calibration beaker. Make sure that there are no air bubbles in the membrane. Measure a second calibration solution and proceed to the next calibration solution.
        3. Rinse the probe with deionized water and place it in a third calibration beaker. Make sure there are no air bubbles in the membrane. Measure the third calibration solution.
        NOTE: When the calibration is good, the display will briefly show, "3rd Buffer OK" and will then return to the main menu.
    4. Use the buffers as follows for ATPase staining.
        1. To prepare a solution at pH 10.3, use two different solutions: (A) 4.506 g of glycine, 4.8 g of $CaCl_2$, 3.51 g of NaCl, and 600 mL of dH_2O and (B) 2.176 g of NaOH and 540 mL of dH_2O. Store the solutions in a cold room or a refrigerator. Use them within one month.
        2. To prepare solutions at pH 4.3 and 4.6, perform "acid preincubation." Prepare the acid for preincubation using: 6.47 g of Na acetate, 3.7 g of KCl, and 500 mL of dH_2O. Thereafter, prepare 1% $CaCl_2$ solution by dissolving 2.5 g of it into 250 mL of dH_2O. Prepare 2% $CoCl_2$ solution by dissolving 5 g of it into 250 mL of dH_2O.
        3. Store and use these solutions as mentioned above. Finally, prepare 0.2% ammonium sulphide by mixing 800 µL of 20% $(NH_4)_2S$ into 40 mL of dH_2O. Prepare the latter freshly.
    5. Prepare solutions at certain pH values as follows. After the calibration of the pH-meter, remove the cuvettes and calcium and cobalt chlorides from refrigerator and allow them to warm to room temperature before staining.
        1. For **pH 10.3**, add around 25 mL of solution A to a small (approximately 70 mL) glass beaker. Measure the pH. Keep adding solution B until the required pH of 10.37 is reached. If the staining is too dark, increase the pH. If it is too bright, reduce the pH.
        2. For **pH 4.6**, add around 25 mL of "acid preincubation" to a small glass beaker. Measure the pH. Reduce the pH using 5 M acetic acid. If the image of the stain is too dark, try to lighten with increased pH. If it is too bright, darken with a decreased pH. If staining does not help, try another pH: 4.8 instead of 4.6.
        3. For **pH 4.3**, do the same as for 4.6, but add more acetic acid. Decrease the pH if the stain is too bright, and increase the pH if it is too dark for the fibers to be specified.
        4. Prepare ATP solution as follows. Weigh 0.017 g of ATP per cuvette (10 mL), so 0.051 g per 3 cuvettes or 0.068 g for 4 cuvettes. Take 30 mL (for 3 cuvettes, 10 mL/cuvette) of solution at pH 10.3 (use a cylinder scale glass) and put it into a glass beaker with weighed ATP.
            1. Mix thoroughly and measure the pH. Reduce the pH using concentrated HCl until the pH reaches exactly 9.40.
        5. For incubation at various pH values, do the following. Place 10.3 solution into one cuvette and incubate it in a water bath at 37 °C for 9 min. Place 4.3 solution into another cuvette and incubate it at room temperature for 5 min. Place 4.6 solution into the last cuvette and incubate at RT for 1 min.
        6. Following the preferred pH incubation procedure, apply the contents of each cuvette as follows. Wash 15 times with dH_2O. Add ATP solution (0.170 g of ATP/100 mL of H_2O) to the biopsy sample. Incubate in a water bath at 37 °C for 30 min. Wash 15 times with dH_2O.
        7. Add $CaCl_2$ solution (1 g of $CaCl_2$/100 mL of H_2O) to the biopsy sample in the cuvettes. Incubate at RT for 3 min. Wash 15 times with dH_2O. Add $CoCl_2$ solution (2 g of $CoCl_2$/100 mL of H_2O) to the biopsy sample in the cuvettes. Incubate at RT for 3 min. Wash 15 times with dH_2O.
        8. Put it in $(NH_4)_2S$ solution for 30 s and wash quickly 15 times under the fume hood. Glue the biopsy slices onto slide glass. To avoid bubbles, squeeze the biopsies, but not too hard.
    6. Select one region of the cross-section without artifacts or longitudinal cuts of the fiber. Analyze under a light microscope using software.

7. Assess the cross-sectional area (CSA), capillaries, and classification of fiber type (*i.e.,* type-I, IIA, or IIX) via computer image analysis from a mean of at least 150-200 fibers per biopsy. From a microscope picture of muscle fibers in the cross-sections ensure that the three types of muscle fibers (*i.e.,* type-I, IIA, and IIX) have various shades of white to gray to black, depending on the pH staining (*i.e.,* 4.34, 4.65 and 10.37).
8. Start by marking some type-I fibers. Thereafter, the program will automatically register the other type-I fibers. Check that all type-I fibers are marked correctly. To mark a certain fiber, click the "Vector" button. Use the cursor to measure the area for each individually selected muscle fiber.
9. After the analysis of type-I fibers, continue the same procedure for type-IIA and type-IIX. The average ± SEM for each type of muscle fiber (*i.e.,* type I, IIA, and IIX) should be calculated regarding amount of fiber and the CSA for the RET and CON groups.
   Note: The cross-sectional area (CSA), capillaries, and classification of fiber type (*i.e.,* type I, IIA and IIx) were assessed from a mean of 163 ± 9 fibers per biopsy.

Representative Results

Material

In the study, 21 relatively healthy women and men, 65-80 years old and with BMI values between 20 and 30 $kg \cdot m^{-2}$ participated and were randomized into two groups. Individuals in both groups had relatively low physical activity levels (*i.e.,* a moderate everyday physical activity level and no regular exercise training). One group (n=12, 6 women and 6 men) performed RET under a trainer for 1 h three times a week for eight weeks, and the other group served as controls (n=10, 5 women and 5 men). The RET and CON groups were balanced in terms of age, sex, and BMI (**Table 1**). More subjects were recruited to the RET group to make up for dropouts; more were anticipated in the RET group over the CON group.

		RET (n=12)		CON (n=9)	
		Pre	**Post**	**Pre**	**Post**
Age (years)		71.4±1.1		72.0±1.4	
BMI		24.6±0.8	24.9±0.8	23.2±0.8	23.2±0.8
Weight (kg)		70.4±2.9	71.1±2.8	67.4±3.9	67.6±3.9
FFM (kg)		51.0±2.3	52.4±2.1**	47.6±4.1	48.6±4.3
Thigh Cross-sectional area (cm^2)		188.9±9	200±8***†	155±12	154±11
Fiber Cross-sectional area (cm^2)	**Type I**	5452±393	5567±362	4889±323	4807±354
	Type IIa	4230±610#	4484±434#	4114±535#	3971±494#
	Type IIx	3678±634#	3554±552#	3392±889#	2913±427#

Table 1: Participants' Characteristics. RET, resistance exercise training; CON, control; BMI, body mass index; FFM, fat-free mass. Values are from 12 (RET) and 9 (CON) subjects, except for fiber cross-sectional area (RET, n=10; CON, n=7), and are presented as the mean ± SEM. **, p<0.01 versus pre; ***, p<0.001 versus pre; †, p<0.05 versus CON post; †††, p<0.001 versus CON post; #, p<0.05 versus type I. This table has been modified from Frank *et al. Scand. J. Med. Sci. Sports*. 2016:26, 764-73.[28]

Beta-blocker users and those with coronary artery disease and severe neurological or joint problems were excluded. At baseline, some subjects had: high blood pressure (2 in each group); depression (1 in each group); and medication for dyslipidemia (2 in RET and 1 in CON), hypothyreosis (1 in RET), an early stage of Parkinson's disease (RET). Medication was taken sporadically for asthma (1 in RET) and rheumatic problems (1 in CON). One person had a pacemaker (CON).

One RET subject interrupted the training after 6 weeks due to back pain but was still included in the study. One initial CON subject was excluded due to knee problems during the pre-test of strength. Those with asthma and the pacemaker were excluded from the cycle test.

The subjects gave their written consent after have been informed of possible discomfort and risks in the test and training sessions.

Data are presented as means ± SEM. Differences between RET and CON were tested for statistical significance with two-way repeated measures ANOVA using a statistical program. When significant main effects or interactions were shown, differences were located with post-hoc analyses (Fisher LSD). Statistical significance was accepted at $p<0.05$.

The trainees (RET) showed, compared to the CON group, improvement on the measurements taken in strength, power, glucose tolerance, and several parameters of muscle aerobic capacity. Using an isokinetic dynamometer for knee extensor strength permitted the measurement of concentric, eccentric, and static strength (which all increased by 8-12% for RET post- versus pre-test, **Figure 2A**). The dynamometer also showed the rate of force development (RFD), with an increase of 52% (at the initial 0-30 ms) for the RET group (**Figure 2B**). For the CON group, concentric strength was reduced during the intervention period. The training load for RET improved by 19-72% for the training exercises performed.

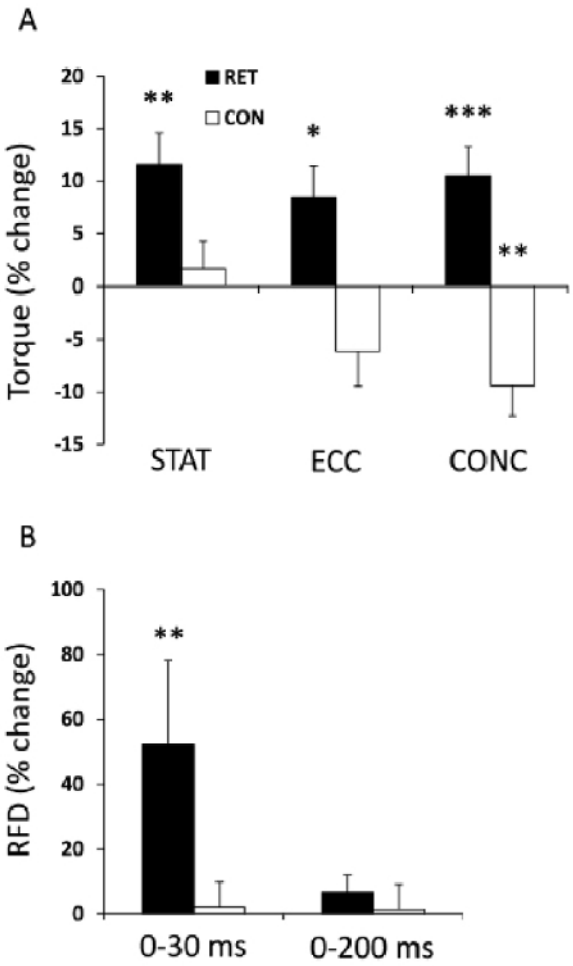

Figure 2: Strength measurement results. The effect of resistance exercise training (RET) or control period (CON) on (**A**) static (STAT), eccentric (ECC), and concentric (CONC) torque and (**B**) rate of force development (RFD) during 0-30 ms and 0-200 ms of static knee extension. Values are from 12 (RET) and 9 (CON) subjects and are presented as percent change relative to basal values (mean ± SEM). *, $p<0.05$ versus pre; **, $p<0.01$ versus pre; ***, $p<0.001$ versus pre. This figure has been modified from Frank *et al. Scand. J. Med. Sci. Sports*. 2016: 26, 764-73.[28] Please click here to view a larger version of this figure.

From the muscle biopsy samples, histochemistry indicated that the amount of fiber type IIa increased, and there was a trend to a decrease in IIx for the RET group. Thus, the RET group showed a change to a more oxidative profile in terms of fiber composition (**Figure 3**). Note that reliable cross-sections could not be obtained from the biopsies of four subjects (two from each group), and the results from these subjects were excluded.

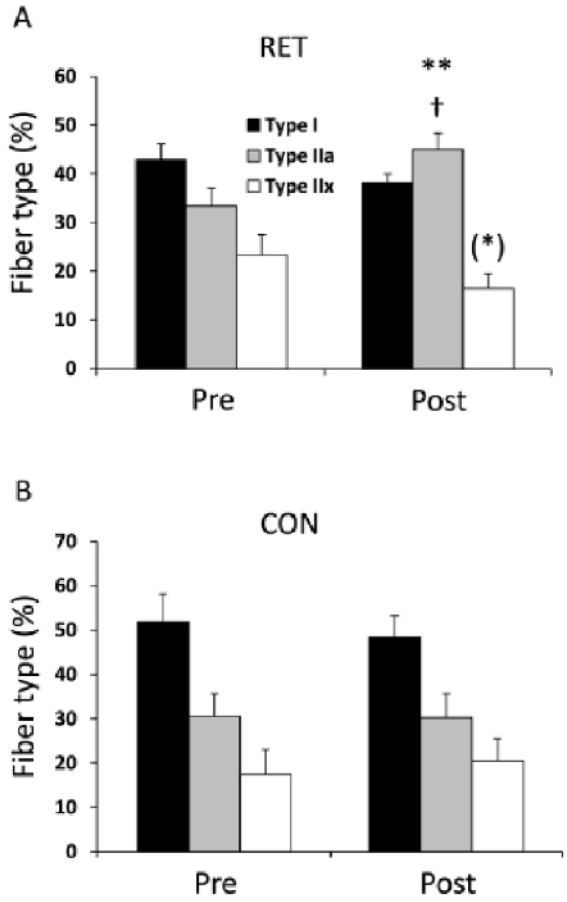

Figure 3: Muscle fiber type composition results. The effect of resistance exercise training (**A**, RET) or control period (**B**, CON). Values are from 10 (RET) and 7 (CON) subjects and are presented as the mean ± SEM. (*), p=0.068 versus pre; **, p<0.01 versus pre; †, p<0.05 versus CON post. This figure has been modified from Frank *et al. Scand. J. Med. Sci. Sports*. 2016: 26, 764-73.[28] Please click here to view a larger version of this figure.

Furthermore, Western blot analyses for determining protein content related to the signaling of muscle protein synthesis showed a rise of 69% for both Akt and mTOR (mammalian target of rapamycin) among the RET group (**Figure 4A** and **Figure 5**). Western blot analyses also proved, among mitochondrial proteins, an increase of about 30% both for OXPHOS complex II and citrate synthase, and of 90% for complex IV in the RET group (**Figure 4B** and **Figure 5**). The primary antibodies used were mTOR, Akt, and OXPHOS. Anti-rabbit or anti-mouse HRP was used as THE secondary antibody. The protein bands for OXPHOS complex I were not clearly visible, and these data were discarded.

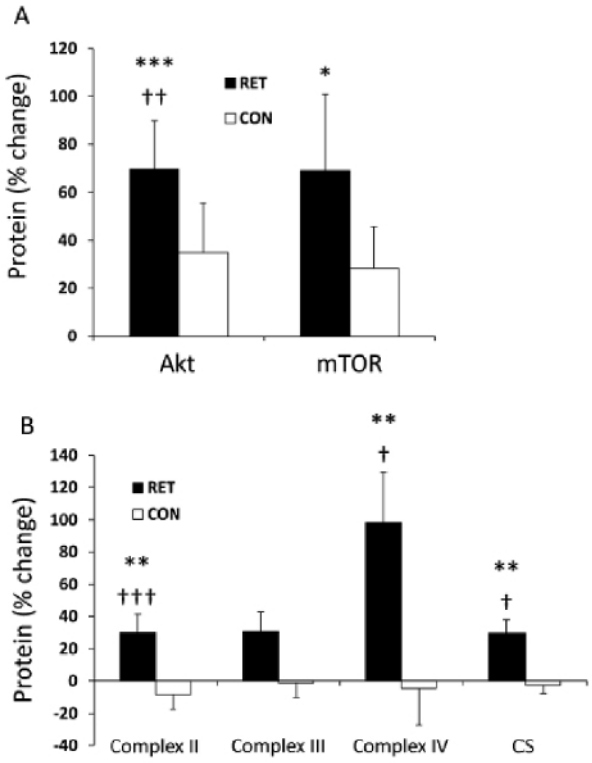

Figure 4: Muscle protein results. The effect of resistance exercise training (RET) or a control period (CON) on changes in muscle contents of Akt and mTOR proteins (**A**) and mitochondrial proteins (**B**). Akt, protein kinase B; mTOR, mammalian target of rapamycin; CS, citrate synthase. Values are the means ± SEM from 11 (RET) and 9 (CON) subjects. *, p<0.05; **, p<0.01; ***, p<0.001 versus basal. †, p<0.05; ††, p<0.01; †††, p<0.001 versus CON post. This figure has been modified from Frank *et al. Scand. J. Med. Sci. Sports*. 2016: 26, 764-73.[28] Please click here to view a larger version of this figure.

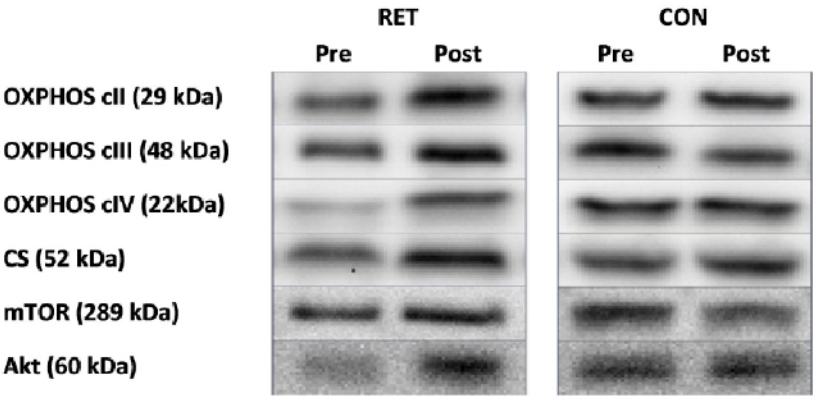

Figure 5: Western blot images. Measured muscle protein before and after eight weeks of intervention. Representative images from one subject in the RET and CON groups, respectively. This figure has been modified from Frank *et al. Scand. J. Med. Sci. Sports*. 2016: 26, 764-73.[28] Please click here to view a larger version of this figure.

Only the RET group showed an increased aerobic capacity in the cycle test (post- versus pre-test). At the highest submaximal intensity, the heart rate (HR) showed a strong trend to decrease in the RET and rise in the CON group (**Figure 6A**). In addition, RER (respiratory exchange ratio = CO_2/O_2) was significantly reduced for the RET group only (**Figure 6B**).

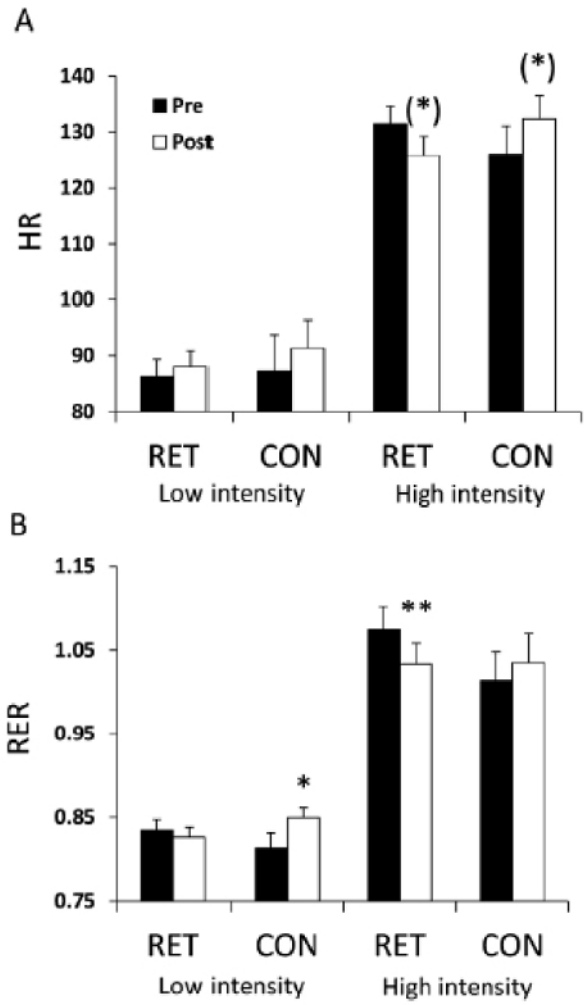

Figure 6: Cardio respiratory data. Pre- and post-resistance exercise training (RET) or control period (CON). (**A**) HR, heart rate and (**B**) RER, respiratory exchange ratio during low- (30 W) and high- (60-120 W) intensity steady-state cycling. Values are from 11 (RET) and 8 (CON) subjects (two subjects were excluded due to asthma and the use of a pacemaker) and are presented as the mean ± SEM. (*) p=0.056 (RET) and p=0.068 (CON) versus pre; *p<0.05 versus pre. This figure has been modified from Frank *et al. Scand. J. Med. Sci. Sports*. 2016: 26, 764-73.[28] Please click here to view a larger version of this figure.

The RET group's results from the glucose tolerance test showed improved blood glucose, both in blood values after 2 h (14%) and for the area under the curve (21%, **Figure 7A**).

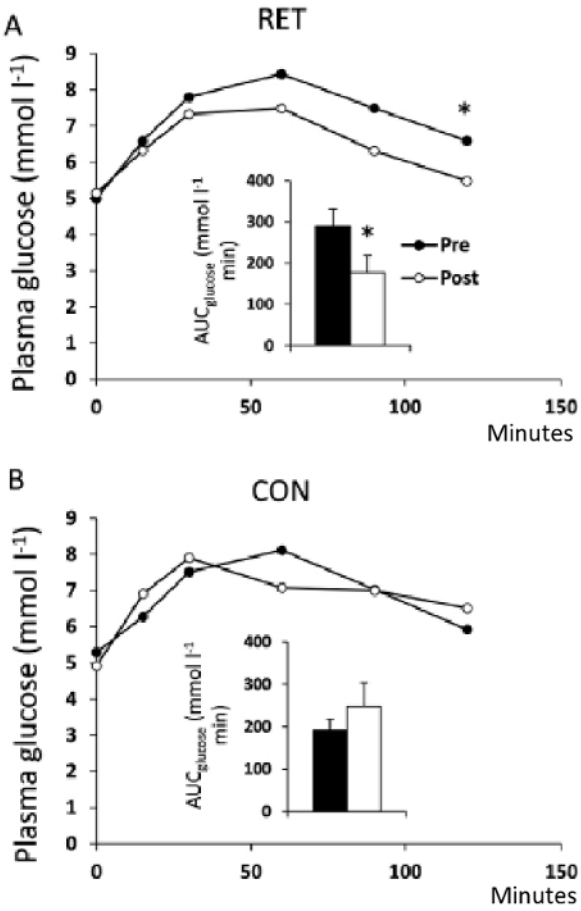

Figure 7: Plasma glucose during OGTT. The test was performed pre- (●) and post- (○) resistance exercise training (RET, **A**) or a control period (CON, **B**). $AUC_{glucose}$, area under the curve for plasma glucose. Values are from 12 (RET) and 9 (CON) subjects and are presented as the mean (plasma glucose) and mean ± SEM ($AUC_{glucose}$). *p<0.05 versus pre. This figure has been modified from Frank *et al. Scand. J. Med. Sci. Sports*. 2016: 26, 764-73.[28] Please click here to view a larger version of this figure.

The blood lipid profile improved for the RET group, with a decrease in apolipoprotein B (8%). For CON, an increase was found (10%). Furthermore, the fat-free mass (FFM) increased by 3% and the thigh cross-sectional area (CSA) by 7% for the RET group (**Table 1**). The assessed improvements seen after the short period of progressive strength training in mitochondrial function, aerobic capacity, glucose tolerance, muscle strength, and power are highly desirable health effects in an elderly population.

The eight strength training exercises are shown in **Figure 8**. Every training task was performed 12 times in each of three sets in every training session 3 times a week for eight weeks.

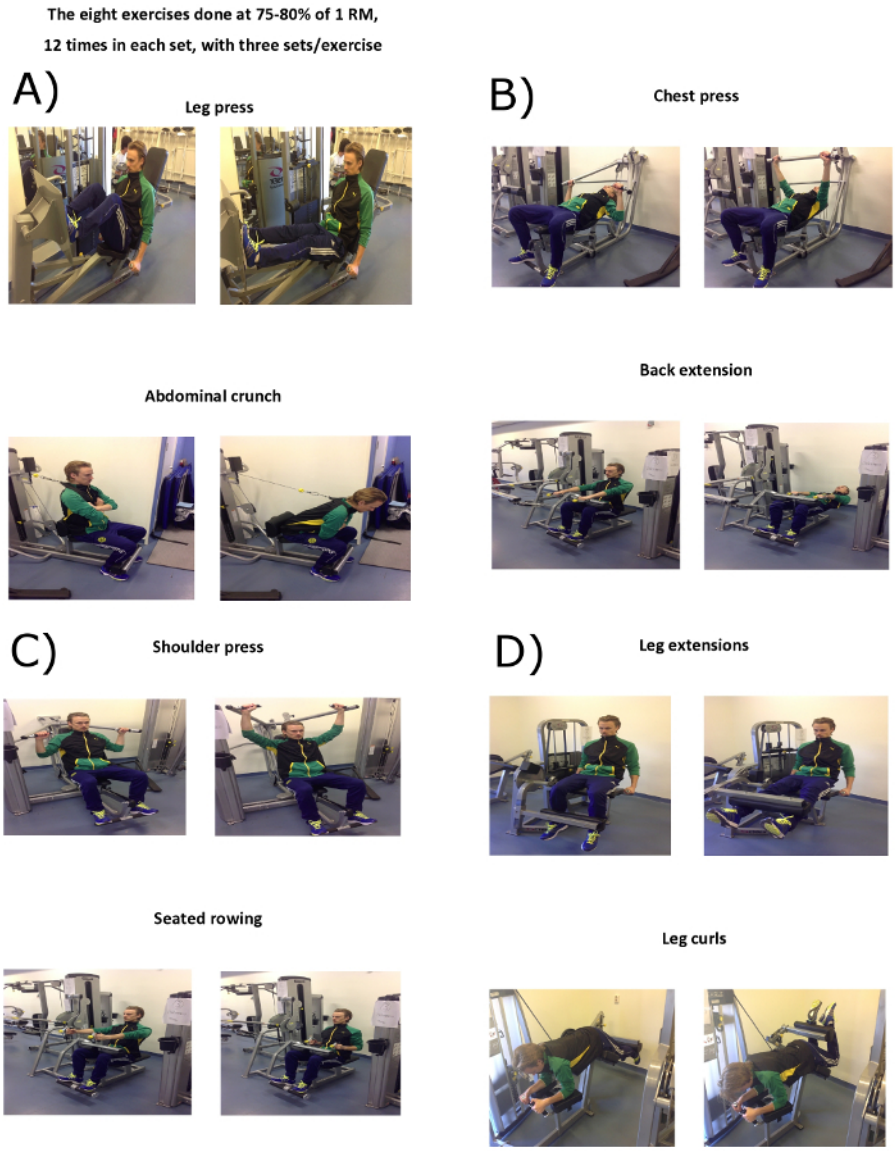

Figure 8: The eight training exercises. The exercises were performed at 75-80% of 1 RM, 12 times/set, with three sets/exercise and training session. The exercises were: "leg press" and "abdominal crunches" (**A**), "chest press" and "back extensions" (**B**), "shoulder press" and "seated rowing" (**C**), and "leg extensions" and "leg curls" (**D**). The range of motions in the strength training exercises are shown here. In the seated abdominal crunch, the trunk should be moved from upright position to 60° forward trunk flexion. In seated back extension, the trunk, from an almost upright seated position, is moved backwards to a horizontal lying trunk position. Both the seated exercises, leg presses and leg extensions, were performed starting with the legs in 90° of knee flexion and ending just before the legs were straightened (near 0° in the knees). Leg curls (in the prone position) where done from nearly straightened legs to approximately 100° of knee flexion. Both the seated exercises, chest press and shoulder press, were performed from 90° elbow flexion to just before the arms were straightened (near 0°). Please click here to view a larger version of this figure.

Discussion

In this study, a number of techniques have been used to investigate the effects of short-term progressive resistance training on elderly subjects' muscle function/morphology, aerobic capacity, and glucose tolerance. The main finding was that, compared to a control group, many improvements occurred in muscle aerobic capacity, glucose tolerance, strength, power, and muscle quality (*i.e.,* protein involved in cell signaling and muscle fiber composition). An increase was, for example, seen for: static, eccentric, and concentric maximal knee extension strength (8-12%); the training loads (19-72%), maximal rate of force development (RFD) at the initial 0-30 ms (52%); several mitochondrial proteins (30-90%); the proteins Akt and mTor, involved in the muscle protein synthesis (both 69%).

Elderly people can have difficulties with sustained health during such a project. One must be aware of the risk for various injuries due to testing and training among untrained elderly. One person in the RET group at the end of the training period had a relapse of former back problems. However, no injury or discomfort occurring during the training project remained for a prolonged time after the end of the investigation among the

old participants. Modifications can sometimes be done regarding when, how much, and how intensively the training should be done. Regarding the strength training regime, it is preferable that the coach registers the load obtained for each training exercise and subject at each training session so that a proper progression can be followed throughout the period. During strength measurement with the isokinetic dynamometer, it is important to avoid any error in the measurement procedure so that the elderly subjects do not miss their maximal performance during their trials. For this reason, it is of value to have warm-ups. Use 8-10 min of ergometer cycling at submaximal levels prior to the strength measurements, followed by initial trials as a familiarization procedure in the dynamometer for knee strength recordings. Furthermore, it is a good idea to perform four recordings during the recordings of each type of muscle strength contraction; highest value found can be selected. It is also of great value to examine the modification of strength assessment in relation to velocity when achieving the test parameter power. In particular, increased power is an important factor for improved health among elderly people. Regarding the biopsy, the subjects are told to avoid aspirin or other anti-coagulation agents before and after the biopsy. Concerning the determination of muscle fiber area in duplicate biopsies from the same leg for type I, type 2A, and type 2B, the reported errors are about 10, 15, and 15%, respectively[29]. This must be considered when evaluating such analysis from a muscle biopsy.

The limitations include concerns regarding Western blot; the method gives no information about protein localization and is highly dependent upon the specificity and quality of the antibody (a major issue). The multi-step analysis increases the risk of errors and aggravates troubleshooting. However, there are several advantages of Western blotting: it is relatively cheap and fast; it gives a high data output in relation to the amount of tissue required; one acquires information about protein expression and protein size; and finally, the coefficient of variation is generally less than 5%. The period of strength training was only eight weeks, and no later follow-up measures have been done with these elderly people. The glucose tolerance tests based on drinking glucose solutions (OGTT) are not considered as appropriate as when the glucose is injected directly into the blood. However, the method used with OGTT is cheaper, easier to administrate, and is widely used in the clinic. Regarding the strength measures with the isokinetic dynamometer, only muscles contributing to knee extensor strength were studied, and not the other major body muscle groups.

In addition to improved strength, resistance training also improved glucose tolerance and muscle oxidative capacity. There were large increases in the training load for each exercise performed (19-72%), demonstrating that resistance training afforded substantial improvements in overall strength. Measurements with an isokinetic dynamometer provided more detailed information on knee extensor function. The torque during static, eccentric, and concentric contraction increased by 8-12%. Furthermore, resistance training resulted in a large increase (52%) in the rate of force development (RFD) during the initial phase of contraction (0-30 ms), whereas it was unchanged between 0-200 ms. The training protocol was well tolerated and, contrary to our expectations, there were no dropouts in the RET group.

Resistance training resulted in hypertrophy, measured as increases in FFM, thigh circumference, and thigh cross-sectional area. The CSA of the different muscle fiber types was not changed significantly after the RET, but there was a shift in fiber type composition from type IIx to type IIa. Since type IIa fibers are larger than type IIx fibers, this contributed to the increased muscle mass. In the RET group, this indicates that protein synthesis was enhanced. The underlying molecular signaling pathway for protein synthesis involves the activation of Akt and mTOR. Elderly people have less mTOR protein in muscle[30], which may restrict protein synthesis. An interesting novel finding is the increased protein levels of mTOR and Akt in the RET group. The observed increase in mTOR here may counteract any possible anabolic resistance and contribute to increased protein synthesis.

VO_{2max} or, more correctly, VO_{2peak}, is often assessed as the maximal VO_2 measured during a test where the work rate is increased step-wise until exhaustion. However, in aged, frail subjects, it is problematic to use exhaustive exercise tests. One problem is that it is not uncommon that the elderly have a latent cardiovascular disease which, during an exhaustive exercise test, leads to an increased risk of heart attack. Another, more technical, problem is that reduced muscle strength rather than a cardiorespiratory limitation may limit the work rate during incremental exercise. Interpretation of data will, under these conditions, be more complicated. An alternative method, used in this study, is to measure HR and RER at a fixed work rates pre- and post-intervention. The results showed that the HR tended to decrease in the RET but increase in the CON group. This suggests that strength training improves VO_{2max} and endurance exercise capacity. These findings match with the results in some[9,31], but not all[32], previous studies. Furthermore, several findings in this study show that muscle aerobic capacity improves (*i.e.,* with changes to a more oxidative fiber type composition and increases in a number of mitochondrial proteins). Although it is well known that endurance exercise improves muscle aerobic capacity in the elderly, studies of strength training give a more contradictory view[8,9,10,33]. Differences in initial training status and training programs may explain the different outcome in different studies. The present results showing a robust increase in several mitochondrial proteins after only eight weeks of training (previous intervention periods were >12 weeks) demonstrate that resistance training can be an effective strategy to improve muscle oxidative capacity.

Despite the short intervention, improved glucose tolerance was observed in the RET group, as shown by the reduction in $AUC_{glucose}$ and $GLU_{120\ min}$. Although obesity and physical inactivity are factors associated with an increased risk of insulin resistance and type 2 diabetes, the molecular mechanisms remain obscure. The altered body composition with increased muscle mass will likely contribute to the improved glucose tolerance in the RET group. Furthermore, it has been hypothesized that insulin resistance is linked to a sedentary lifestyle, with excess lipid supply leading to lipotoxicity, mitochondrial dysfunction, and oxidative stress[3]. The present study shows that resistance training results in a robust increase in mitochondrial oxidative proteins. We hypothesize that the increased muscle oxidative capacity is one factor explaining the increased glucose tolerance.

Investigations with longer follow-ups are desirable for showing whether and for how long the health effects persist in terms of improved muscle aerobic capacity, strength, power, glucose, and lipid values. Also, it is of value to determine the sufficient dose of regular strength training among elderly people. Future applications are also strength measurements in major muscle groups other than the knee extensors. One can also make several other detailed analyses within the muscle cells regarding various proteins and functions within and without the mitochondria.

It is important to have one day in between each test day with no vigorous or prolonged physical activity, the same day or the day before the tests, since this can influence the outcome of the assessments. Examples of critical steps regarding histochemistry and ATPase staining for fiber type composition include ensuring that the piece from the biopsy is treated with isopentane shortly after the biopsy is taken and that the isopentane is at the right temperature so that the biopsy will not be destroyed. Furthermore, the biopsy piece must be "stretched or installed," so that the fibers are pointing in the same direction, prior to treatment with isopentane. During staining, the pH and temperature of the laboratory must be optimal

(and this is difficult to predict). However, this is the only way to ensure the fiber types and fiber area. In addition, the method is quick, showing results within two days, and the technique is relatively inexpensive, with no costly chemicals or devices needed.

The distinct improvement in muscle aerobic capacity after strength training challenges the view that endurance exercise is the preferred mode of exercise. However, in elderly people with low VO_{2max} and muscle strength, endurance exercise must be performed at low intensities. One of the main stimuli of mitochondrial biogenesis is muscle energetic stress[34]. Strength training induces a major local energetic stress, whereas this is less prominent during low-intensity endurance exercise. We hypothesize that in elderly people, strength training is more efficient than endurance exercise to enhance muscle aerobic capacity. Furthermore, considering the improvements in a number of health-related parameters and the high compliance, strength training may be recommended for elderly people.

Disclosures

The authors declare that they have no competing financial interests.

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