Quantitative Ultrasonography of Muscle: Detection of Adaptations to Training in Elderly Women

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Objective: To develop quantitative ultrasonography in studying the adaptation of quadriceps muscle mass and composition to short-term physical training and rehabilitation in elderly women

Design: Randomized control trial.

Setting: Measurements in a research laboratory and training in a fitness center and sports hall.

Participants: Forty-two women, aged 76 to 78 years, with no indications against intensive physical exercise, randomly assigned to strength (n = 16), endurance (n = 15), and control (n = 11) groups. Twelve subjects from the strength, 12 from the endurance, and 11 from the control group completed the study.

Intervention: Supervised physical training 3 times a week for 18 weeks. Strength training: 3 to 4 sets of 8 to 10 repetitions with a load of 60% to 75% of the subjects' 1-repetition maximum. Endurance training: track walking and step aerobics at an intensity of 50% to 80% of maximum heart-rate reserve.

Main Outcome Measures: Ultrasonographic measurements of the quadriceps cross-sectional area (CSA), thickness, and weighted mean grey shades of vastus lateralis (MGS $_{\rm VI}$) and femur (MGS $_{\rm E}$).

Results: No significant interaction of group by time was observed in any of the outcome measures. The changes observed in CSA in the strength group correlated with those measured by computed tomography reported earlier. MGS_{VL} decreased and MGS_F increased in the strength group, suggesting a decreased proportion of fat in the muscle.

Conclusions: Quantitative ultrasonography is a potentially useful tool for studying skeletal muscle in elderly women. The precision and accuracy of the method, however, should be improved to reveal the adaptation of aging muscles to short-term physical training and rehabilitation programs.

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EARLIER CROSS-SECTIONAL studies have shown B-mode ultrasonography to be a valuable research tool for skeletal muscle mass and composition in comparing children with neuromuscular disease with healthy controls^{1,2} and in comparing elderly male and female athletes with untrained men and

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women.^{3,4} Ultrasound scanning has also been used in studying the muscles of elderly men⁵ and women^{6,7} in comparison with those of younger people. Short-term changes in aging skeletal muscle, such as the effects of physical training and rehabilitation programs have, however, been much less frequently evaluated using ultrasound imaging. The exercise interventions reported so far have only focused on relatively young men and women.⁸⁻¹⁰

Ultrasound scanning is advantageous compared to the other medical imaging modalities such as computed tomography (CT) because it is nonionizing in nature and is thus usable in children and in longitudinal studies in which several measurements are to be performed. Ultrasound scanners are also less expensive when compared to CT and nuclear magnetic resonance imaging.

Ultrasound scanners enable rather specific measurements of muscle cross-sectional area (CSA) and thickness, which means that bone, subcutaneous fat, and larger connective tissue structures can be excluded from the analysis. The echogenicity pattern of ultrasound scans has been evaluated to obtain more information about muscle composition. In those studies, ultrasound scans have been assessed by blind observers, and the data have been more or less descriptive. There are few studies reporting the development of quantitative ultrasonography analyses to evaluate muscle composition. Cady et al. measured the back-scatter amplitude of the quadriceps femoris using a real-time scanner. Heckmatt et al. measured the echo amplitude reflected from the muscle bulk and from the bone together with the attenuation slope in muscular dystrophy patients.

The purpose of this study was to apply quantitative ultrasonographic analysis to skeletal muscle in elderly women. Specifically, we wanted to know whether it is possible to detect changes in quadriceps muscle mass and composition using ultrasound scanning after short-term, progressive, intensive strength and endurance training in 76- to 78-year-old women.

METHODS

The study was ancillary to an experimental trial on the effects of physical training in elderly women. A detailed description of the study design has been published elsewhere.¹⁵

Subjects

A random sample of 240 women born during 1915-17 was drawn from the population register of the city of Jyväskylä. Of this sample, 64 women reporting no severe diseases or functional impairments were invited for clinical and laboratory examinations. After the examinations the sample was further reduced to 42 women with no indications against intensive physical exercise. The subjects were randomly assigned to strength (n = 16), endurance (n = 15), and control (n = 11) groups.

Twelve subjects from the strength, 12 from the endurance, and 11 from the control group completed the study. Of the 7 women who withdrew from the study, 6 were excluded because of disease and illness unrelated to the exercise, and one was unwilling to continue because of the lack of time in her daily schedule.

The study was approved by the hospital and university ethical

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committees. A written informed consent was obtained in advance from all the subjects.

Anthropometry

Body height, body mass, and lean body mass (LBM) together with body fat measured using bioelectrical impedance were determined. The bioelectrical impedance results of one woman were discarded because of a technical failure. Thigh girth was measured with a flexible tape on the same side and at the same level as the ultrasound measurements.

Ultrasonography

Ultrasound scans were obtained from the quadriceps muscle by selecting the midpoint between the great trochanter and lateral joint line of the knee as the scanning site. The measurements were performed on the side of the dominant hand. During scanning, subjects were in a supine position with the leg extended and relaxed on the examination table. The mid-thigh was marked with an indelible ink for a needle muscle biopsy. The scar was then used as a marker for the 9- and 18-week measurements. The thickness of the subcutaneous fat and the thickness of the muscle group, including the vastus lateralis and vastus intermedius, were assessed by means of a longitudinal real-time scan (Aloka SSD-280 LS, 7.5MHz transducer^b), whereas the CSA of the muscle group was assessed using a compound ultrasound scanner (Aloka SSD-190^b) fitted with a 5MHz transducer. The axial and lateral resolution of the former transducer was 0.4mm and 0.9mm, and that of the latter 0.4mm and 1.6mm. Gain settings and near- and far-field scales were set equally for all subjects and kept constant in all the measurements performed during the experiment.

The composite videosignal was captured from the real-time scanner and the ultrasound image was transferred to the computer for the grey scale analysis using a Digihurst Microeye MT/TC card.c The color monitor of the computer was used during scanning to ensure the best and sharpest bone echo. The probe was then assumed to be at right angles to the femur. The vastus lateralis and the echo reflected from the femur were encircled from the real-time scans (width 3.5cm). A computer image analysis program, which was developed at our university, was used to analyze the grey scale histogram from the encircled areas. Shade 1 represented the black, echo-poor area and shade 64 the most white, high intensity echo area. The weighted mean grey shades of the vastus lateralis (MGS $_{VL}$) and femur (MGS $_{F}$) were then calculated from the histograms (fig 1). Increased echo intensity of the muscle together with decreased intensity of the bone echo was assumed to reflect an increased relative proportion of fat in the muscle.^{3,4} The reproducibility of the ultrasound measurements for muscle thickness and CSA has been reported earlier.3 The coefficient of variation for the ultrasound measurements repeated over 5 days was 7.7% for the MGS_{VL} and 7.3% for the MGS_F. The correlation coefficients were .68 (p < .001) and .88 (p < .001), respectively.

We failed to obtain an acceptable real-time ultrasound scan from one woman in the pretraining measurements. Her data was excluded from the real-time ultrasound analysis. All the other measurements were performed at the beginning of the study and after 9 and 18 weeks of training.

Training

Both experimental groups participated in an 18-week progressive physical training program comprising supervised, 1-hour training sessions 3 times a week.

The strength group trained on machines using compressed

air as a resistance.d These machines resemble the variable resistance type of exercise apparatus, providing both concentric and eccentric resistance that varies in a predefined way throughout the range of motion. This is made to compensate the forcelength relationship of the muscle. The dynamic training was specifically directed at increasing the mass and strength of the quadriceps femoris by means of the leg press and the knee extension, the hamstrings by the leg flexion curl in the standing position, and the calf muscles using the heel raise. Principally, each training session included all the above-mentioned exercises except for the knee extension, which was included in the training program after 9 weeks of training. The resistance was individually adjusted according to the one repetition-maximum test (1 RM) measured at 2-week intervals. The intensity of the training was gradually increased during the 18-week period from 60% to 75% of the 1 RM. The subjects performed 3 to 4 sets of 8 to 10 repetitions with a 30-sec pause between sets. The knee extension was used with caution because it produced discomfort in the knee area in several subjects.

The training of the endurance group included track walking twice a week and step aerobics once a week. The walking distance was increased from the average distance of 1,500 meters to 2,700 meters. During the 40-minute step aerobics sessions, cardiovascular system was stressed by utilizing the major muscle groups of the lower extremities. The stepping height was .10 to .15 meters throughout the 18-week period. The training heart rate was individually adjusted on the basis of a progressive multistage exercise test on a bicycle ergometer. Training intensity was increased gradually during the training period from 50% to 80% of the initial maximal heart-rate reserve. The training heart rates were controlled by heart-rate monitors throughout the training sessions.

The mean participation in the strength training sessions varied between 71% (knee extension) and 86% (leg press), compared to an average attendance of 87% in the endurance training sessions. ¹⁵ The main reasons for nonparticipation in a training session were a trip abroad and acute respiratory infection.

The controls were instructed to continue their daily routines and not to change their physical activity levels.

To determine the actual physical activity level of the subjects and possible changes in it during the experiment, all the subjects were instructed to keep a diary recording their daily physical activities according to the type and duration of physical activity performed. This included recording kilometers for walking, cycling, and swimming. Beyond the training included in the trial the study groups did not differ with respect to the overall level of physical activity, which remained constant throughout the experiment.

Statistical Analysis

Standard procedures were used to calculate means, standard deviations (SD) and correlation coefficients (Pearson r). The differences between the study groups in the baseline measurements were assessed using one-way analysis of variance (AN-OVA). The effects of the training programs were assessed using sphericity-corrected ANOVA for repeated measures. The results were also analyzed by multivariate analysis of variance (MA-NOVA), and these results are also given where notable differences were observed between the results of the two statistical methods. If the significance of the interaction of group by time was p < .10, the training effect was localized utilizing simple contrasts. The level of statistical significance chosen for the contrasts was p < .05. Within-group differences between the three measurements were also assessed using both ANOVA and MANOVA for repeated measures. The level of significance was set at p < .05.

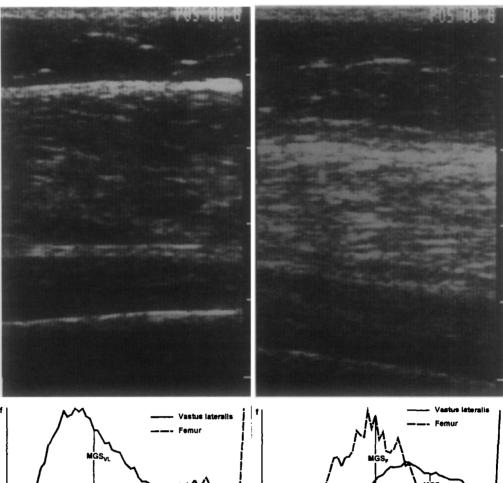
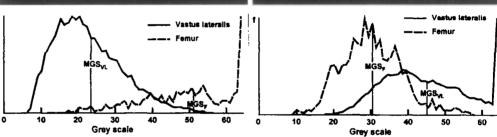


Fig 1. Real-time scans of the vastus lateralis and femur in two elderly women showing markedly different echogenicity patterns and grey scale histograms. The right scan indicates an increased proportion of fat in the muscle. MGS_{VD}, mean grey shade of vastus lateralis: MGS. mean grey shade of femur.



RESULTS

In the baseline measurements, the study groups did not differ with respect to any of the physical characteristics or quadriceps muscle properties under investigation.

The physical characteristics of the subjects who completed the study are listed in table 1. The strength-trained women increased their LBM and decreased their body fat when compared to the changes observed in the controls. When localized more specifically, the changes were significant after the first 9 weeks of training (p = .005 and p = .029, respectively).

Comparison of the baseline, 9-week, and 18-week measurements within the study groups showed a significant decrease in body mass and in the thickness of subcutaneous fat in the midthigh in the strength (p = .004 and p = .022) and endurance groups (p = .004 and p = .002, respectively). The control group showed a significant decrease in thigh girth (p = .031) during the experiment.

The results for the quadriceps muscle mass and mean grey shades are shown in table 2 and figures 2 through 6. When the present variables were related to the CT measurements reported earlier, 15 there was a significant correlation between the quadriceps muscle CSA measured by the two techniques both before (r = .829) and after training (r = .916) and between the MGS_F and fat area inside the quadriceps muscle before (fig 2) and after training (r = -.615).

There was an overall change in thickness, CSA, and MGS_{vL} over time (table 2). No significant interaction of group by time, however, was observed for muscle thickness, CSA, MGS_{vL}, or MGS_F.

When comparing the baseline, 9-week, and 18-week measurements within the groups (ANOVA), the decrease in the CSA of the quadriceps was significant in the endurance-trained (p = .002) and in the control (p = .029) women. In the strengthtraining group, the MGS_{vi.} decreased (ANOVA p = .070, MA-NOVA p = .007) and the MGS_F increased (ANOVA p = .122, MANOVA p = .012) during the experiment.

When considering the individual differences in quadriceps CSA before and after 18-weeks of training, the average change was 0.7% in the strength-trained, -5.3% in the endurancetrained, and -7.1% in the control women (fig 3). The change observed in the CSA measured by ultrasound in the strength group tended to correlate with that measured by CT (fig 4).

The changes in MGS_{VL} were on average -9.0% in the strength group, -1.4% in the endurance group, and 1.0% in the controls (fig 5). The respective changes in MGS_E were 14.4%, 2.7%, and 2.2% (fig 6).

DISCUSSION

In the present study, quantitative ultrasonography was applied to examine the changes in muscle mass and composition of quadriceps femoris after short-term physical training program.

Table 1: Physical Characteristics in 76- to 78-Year-Old Women Before and After 9 and 18 Weeks of Intensive Strength and Endurance Training (Mean, SD)

	Str	Strength (S) $(n = 12)$	2)	Endui	Endurance (E) $(n = 11-12)$	1-12)	3	Control (C) $(n = 11)$		ANONA	ANOVA Significance (p)	(d)	
Variable	Baseline	9-Week	18-Week	Baseline	9-Week	18-Week	Baseline	9-Week	18-Week	Interaction	Group	Time	Contrasts
Body height (cm) Body mass (kg)		159.6 (3.5) 66.5 (9.2)	159.9 (3.3) 65.3 (9.4)	156.7 (5.5) 67.3 (9.6)	156.6 (5.2) 66.6 (9.2)	156.9 (5.4) 65.9 (9.1)	158.7 (5.6) 67.6 (12.8)	158.7 (5.5) 66.9 (13.0)	159.1 (5.6) 66.7 (13.4)	.942	.329	^ ^ 100. 100.	
LBM (kg)	45.3 (3.8)	46.1 (2.9)	45.8 (3.1)	44.4 (2.6)	44.6 (2.6)	44.2 (2.8)	45.0 (5.0)	44.5 (5.1)	45.0 (5.1)	.093	989	.749	S-C.040
													S-E .465 E-C .130
Body fat (%)	31.9 (6.4)	29.9 (7.0)	29.2 (7.8)	34.4 (6.2)	33.7 (5.9)	32.9 (5.6)	32.2 (8.0)	32.4 (7.9)	31.2 (7.9)	.030	.526	<.001	S-C.007 S-E.097 E-C.521
Thickness of thigh subcutaneous fat (cm) Thigh girth (cm)	1.23 (.56) 51.0 (5.9)	1.15 (.55) 50.4 (5.4)	1.04 (.42) 50.3 (5.6)	1.14 (.54) 50.8 (4.6)	1.04 (.53) 50.4 (4.2)	1.03 (.48) 50.2 (4.6)	1.34 (.65) 51.2 (6.3)	1.27 (.68) 50.5 (5.7)	1.27 (.61) 50.4 (6.4)	.248 .987	.627 .994	<.001 .003	

Table 2: Effects of Intensive Strength and Endurance Training on Quadriceps Muscle Mass and Echo Intensity Reflected from the Vastus Lateralis and From the Femur in 76- to 78-Year-Old Women (Mean, SD)

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Stre	Ξ	Strength $(n = 11-12)$	6	ū	Endurance $(n = 12)$			Control $(n = 11)$		ANONA	ANOVA significance (p	<u>~</u>
Baseline		9-Week	18-Week	Baseline	9-Week	18-Week	Baseline	9-Week	18-Week	Interaction	Group	Time
2.36 (.36)	i	2.38 (.38)	2.55 (.34)	2.43 (.38)	2.37 (.33)	2.50 (.36)	2.57 (.41)	2.44 (.41)	2.58 (.34)	.485	.736	.003
31.5 (6.2)		32.3 (5.2)	31.3 (4.2)	31.7 (3.6)	30.8 (4.1)	30.0 (3.8)	31.4 (5.1)	30.2 (4.6)	29.0 (4.5)	.196	.725	.004
38.5 (7.8)		37.6 (7.1)	34.6 (6.5)	39.6 (6.3)	41.6 (5.2)	38.8 (5.9)	36.6 (8.3)	36.9 (7.1)	36.1 (5.7)	.417	.323	.036
46.7 (11.0)		50.5 (8.6)	51.3 (7.7)	47.5 (10.0)	48.7 (8.0)	48.1 (8.4)	45.4 (12.1)	47.3 (13.4)	45.4 (11.1)	.377	.693	.078

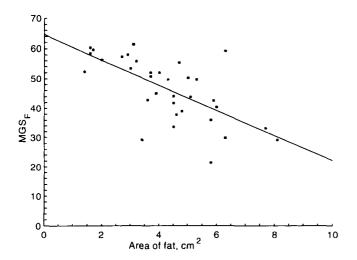


Fig 2. The association between ultrasound-measured mean grey shade of femur (MGS_r) and CT-measured fat area inside the quadriceps muscle $\{r = .665; p < .001\}$

The CSA of the quadriceps remained relatively constant throughout the experiment in the strength-trained women but decreased in the endurance-trained and in the control women during the 18-week period. However, the changes in the quadriceps CSA were not significant when the study groups were compared. In using ultrasound imaging to measure the thickness and CSA of the total muscle compartment, fat that has infiltrated into the muscle, which is observed especially in elderly people, is automatically included in the analyses. Where there is an increase in muscle tissue concomitant with a decrease in fat tissue, possible hypertrophy may not be revealed by measuring the whole muscle compartment. In younger subjects, however, significant increases in quadriceps CSA measured using ultrasound have been reported after only a few months of resistance training. 9.10

In our preceding report, as part of this same study,¹⁵ we observed a significant increase of 4.5% in quadriceps CSA measured by CT in the strength-trained women when compared to

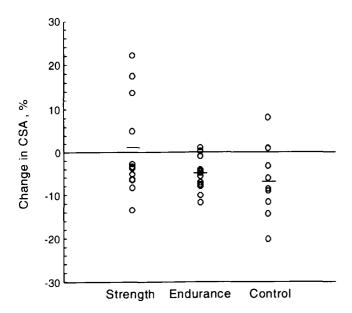


Fig 3. Individual changes in quadriceps CSA in 76- to 78-year-old strength-trained, endurance-trained, and control women (- indicates means).

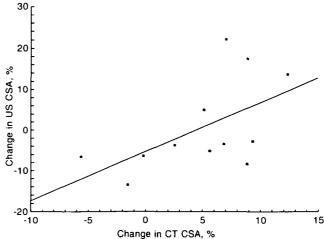


Fig 4. The association between the change in the cross-sectional area (CSA) measured by ultrasound (US) and by CT after 18 weeks of strength training (r = .560; p = .058).

the change (0.2%) in the control women. The difference between the strength-trained and control women was even more pronounced when the lean tissue area of the quadriceps muscle was analyzed (5.8% vs -0.3%, respectively). In light of the CT results, the present observations, especially the relatively large decrease in quadriceps CSA in the controls, are somewhat confusing. It is unlikely that aging could affect muscle mass to that extent during such a short period of time and only when using the ultrasound scanning. We were unable, however, to find any systematic errors in our ultrasound measurements. In agreement with our earlier study⁴ ultrasound scanning yielded, on average, a 30% smaller quadriceps muscle CSA than CT scanning. The correlations between muscle CSA measured by ultrasound and by CT were, however, highly significant. The change in the ultrasound CSA in the strength group also tended to correlate with that in the CT CSA.

When considering the muscle composition of elderly athletes in our earlier studies, ^{3,4} the ultrasound-measured intramuscular echo intensity was lower and the echo intensity reflected from

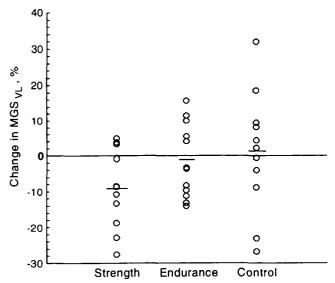


Fig 5. Individual changes in MGS_{VL} in 76- to 78-year-old strength-trained, endurance-trained, and control women (– indicates means).

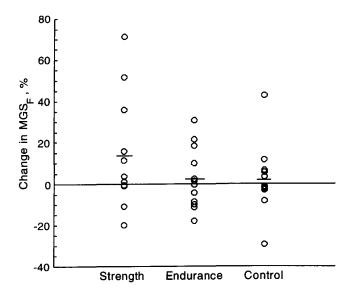


Fig 6. Individual changes in MGS_F in 76- to 78-year-old strength-trained, endurance-trained, and control women (– indicates means).

the femur higher in the athletes when compared to the controls. These differences between the study groups were related to the CT scans, showing a smaller area and lower relative proportion of fat together with a larger lean tissue area inside the quadriceps compartment in the athletes when compared to the controls.⁴

To get more quantitative information about the ultrasoundmeasured muscle composition and training-induced changes in it, grey scale analyses were conducted. In the ultrasound analysis, the MGS_{vI} decreased and MGS_F increased about 10% in the strength-training group. Although these two variables remained relatively constant throughout the experiment in the other study groups, the changes observed in the strength-trained women were not significantly different from those in the endurance or control groups. In our previous CT report from this same study, 15 a significant increase was observed in lean tissue CSA together with a significant decrease (8.7%) in relative proportion of fat due to strength training. The changes obtained by ultrasound and CT techniques did not correlate with each other, although the ultrasound-measured MGS_F correlated with the CT-measured fat area inside the quadriceps compartment both before and after training. This relationship between bone echo and fat area inside the muscle was also observed in our earlier study.4

The reproducibility of the mean grey scale analysis was rather good, although the intensity of the echo reflected, especially from the bone, is sensitive to the inclination of the transducer during scanning. Moreover, during the real-time scanning over the vastus lateralis muscle, satisfactory echoes from the fasciae together with a sharp bone echo were difficult to obtain. Such a difficulty was not observed earlier, 34 when scanning the midthigh more anteriorly above the rectus femoris. However, the

vastus lateralis was scanned because of the needle muscle biopsy, which was also obtained from that muscle.

In conclusion, the results indicate that quantitative ultrasonography is a potentially useful tool for studying skeletal muscle mass and composition in elderly women. However, the precision and accuracy of the method should be further improved to reveal the adaptation of aging muscles to short-term physical training and rehabilitation programs.

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