Impact of Aerobic Exercise Training on Age-Related Changes in Insulin Sensitivity and Muscle Oxidative Capacity

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Insulin resistance increases and muscle oxidative capacity decreases during aging, but lifestyle changes—especially physical activity—may reverse these trends. Here we report the effect of a 16-week aerobic exercise program ($n = 65$) or control activity ($n = 37$) performed by men and women aged 21–87 years on insulin sensitivity and muscle mitochondria. Insulin sensitivity, measured by intravenous glucose tolerance test, decreased with age ($r = -0.32$) and was related to abdominal fat content ($r = -0.65$). Exercise increased peak oxygen uptake (Vo_{2peak}; 10%), activity of muscle mitochondrial enzymes (citrate synthase and cytochrome c oxidase, 45–76%) and mRNA levels of mitochondrial genes (COX4, ND4, both 66%) and genes involved in mitochondrial biogenesis (PGC-1α, 55%; NRF-1, 15%; TFAM, 85%). Exercise also increased muscle GLUT4 mRNA and protein (30–52%) and reduced abdominal fat (5%) and plasma triglycerides (25%). None of these changes were affected by age. In contrast, insulin sensitivity improved in younger people but not in middle-aged or older groups. Thus, the muscle mitochondrial response to 4 months of aerobic exercise training was similar in all age-groups, although the older people did not have an improvement in insulin sensitivity. *Diabetes* 52: 1888–1896, 2003

The number of people with type 2 diabetes and impaired glucose tolerance is rapidly increasing (1,2). Key factors contributing to this increase in diabetes include age, obesity, and sedentary lifestyle (3–8). Exercise is a readily available intervention that can increase insulin action (9–14) and prevent the onset of diabetes (15–18). An important question is whether the effects of aerobic exercise on insulin action are diminished with advancing age. A recent study reported that a vigorous 7-day exercise program increased insulin sensitivity and muscle glucose transporter (GLUT4) content by a similar amount in younger (22 years) and older (61 years) people (12). However, current health and fitness guidelines for healthy adults recommend exercising at more moderate intensities at least 3 days per week over long periods (19). Thus, the first purpose of the current study was to determine whether a 4-month program of bicycle training that could be readily followed by most elderly individuals would lead to a similar improvement in insulin sensitivity in men and women across a wide age span.

Skeletal muscle is the major site of insulin-mediated glucose disposal and is implicated in the pathogenesis of insulin resistance and diabetes (20,21). Several pieces of evidence suggest that insulin action may be related to the oxidative capacity of skeletal muscle. First, aerobic exercise training improves both insulin sensitivity and activity of oxidative enzymes in muscle (22,23). Second, people who are obese and insulin resistant or have type 2 diabetes tend to have lower activity of muscle oxidative enzymes (24,25). Third, insulin infusion preferentially stimulates the synthesis rate of mitochondrial proteins in skeletal muscle (26) and increases the mRNA abundance genes associated with mitochondria and glucose metabolism (27,28). Fourth, recent work has shown that genes for mitochondrial proteins and the primary glucose transporter in muscle, GLUT4, are regulated by common signals, including elevations in cytosolic calcium (29,30) and the transcriptional coactivator PGC-1α (peroxisome proliferator–activated receptor [PPAR]-γ coactivator 1α) (31). Muscle mitochondrial function and gene expression are reduced in aging muscle, but the underlying cause and the relationship to insulin action are not yet understood (32–34). The second purpose of this study, therefore, was to measure the effect of age and exercise training on the gene expression of PGC-1α, GLUT4, and mitochondrial genes and nuclear transcription factors that regulate mitochondrial genes, including PGC-1α, nuclear respiratory factor (NRF)-1, and mitochondrial transcription factor A (TFAM). We tested the hypothesis that a moderate exercise program results in equivalent improvements in insulin sensitivity, GLUT4 expression, and mitochondrial genes and function in individuals whose age ranged between 22 and 87 years.

RESEARCH DESIGN AND METHODS

Participants. Healthy men and women who exercised <30 min twice per week during the previous 9 months were recruited. Health status was assessed by medical history, physical exam, blood chemistries (liver enzymes, creati-

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GCRC, General Clinical Research Center; NRF, nuclear respiratory factor; PGC-1α, PPAR-γ coactivator 1α; PPAR, peroxisome proliferator–activated receptor; TFAM, mitochondrial transcription factor A; Vo_{2peak}, peak oxygen uptake; S_I, insulin sensitivity.

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TABLE 1
Characteristics of the participants at baseline

Variable	Sex	Age-group (years)					
		20–29	30–39	40–49	50–59	60–69	≥70
n	W	7	9	8	6	6	13
	M	8	7	6	5	7	8
Weight (kg)	W	72.2 ± 2.2	66.0 ± 2.4	68.3 ± 2.5	70.1 ± 4.0	73.6 ± 2.0	67.7 ± 2.7
	M	83.2 ± 3.5	88.7 ± 4.1	90.4 ± 3.8	90.4 ± 4.7	92.5 ± 1.9	80.4 ± 4.2
BMI (kg/m^2)	W	24.7 ± 0.7	23.9 ± 0.8	24.8 ± 0.5	25.6 ± 1.2	27.5 ± 0.6	25.7 ± 0.8
	M	25.2 ± 1.0	26.6 ± 0.7	28.2 ± 0.7	27.8 ± 1.2	28.3 ± 0.5	27.1 ± 1.3
FFM (kg)	W	43.4 ± 1.1	39.8 ± 0.9	39.1 ± 0.9	39.6 ± 1.6	40.1 ± 1.5	38.0 ± 1.1
	M	60.7 ± 2.2	60.1 ± 2.7	61.2 ± 3.7	59.1 ± 3.5	61.0 ± 1.2	52.8 ± 1.4
Body fat (%)	W	34.1 ± 2.2	34.1 ± 1.3	37.2 ± 1.3	37.1 ± 2.0	40.3 ± 1.4	38.9 ± 1.5
	M	21.5 ± 1.4	26.7 ± 0.6	26.0 ± 2.9	25.5 ± 0.8	27.5 ± 1.3	26.8 ± 2.2
Abdominal fat (cm^2)	W	201 ± 49	141 ± 16	244 ± 42	206 ± 43	288 ± 53	278 ± 32
	M	230 ± 69	325 ± 32	355 ± 56	352 ± 41	443 ± 34	363 ± 45
Visceral fat (cm^2)	W	45 ± 19	30 ± 4	69 ± 18	51 ± 15	107 ± 26	99 ± 15
	M	100 ± 31	133 ± 13	197 ± 52	172 ± 31	217 ± 31	178 ± 20

Data are means ± SE. Significant differences between men and women were present for all variables. With increasing age, there was a significant decline in FFM and significant increases in percent body fat, total abdominal fat, and visceral fat ($P < 0.01$). Correlation coefficients between these variables and age are shown in Table 2.

nine, electrolytes, and glucose), complete blood count, urinalysis, and electrocardiogram. Exclusion criteria included tobacco use, β-blockers, diabetes or other endocrine disorders, and debilitating chronic illness. Forty-nine women and 41 men between the ages of 21 and 87 years met these criteria and were enrolled after providing written and oral consent. Characteristics of the participants are shown in Table 1. Participants gave their informed oral and written consent before any tests were performed. The Mayo Foundation Institutional Review Board approved the study.

Study protocol. Participants were randomized to either a 16-week aerobic control or exercise program. A similar 5-day protocol was completed at baseline and again within a week of completing the training or control phases. During each study period, a weight-maintaining diet (55:30:15% carbohydrate, fat, and protein, respectively) was provided for the first 4 days. On the morning of day 4, subjects were admitted to the General Clinical Research Center (GCRC) following an overnight fast. Insulin sensitivity and body composition were then measured. The following morning (day 5) blood and muscle biopsy samples from the vastus lateralis (32,35) were obtained.

The exercise program was performed on a stationary bicycle. Training started with three sessions per week, lasting 20 min each, at an intensity eliciting 70% of maximal heart rate. Intensity, duration, and number of sessions were gradually increased so that the final month of training consisted of four sessions per week at 80% of maximal heart rate for 40 min. Exercise specialists supervised each session and recorded heart rates. Compliance with the target workloads and number of sessions was >90%. The exercise protocol was completed by 41 of 47 participants originally assigned. The control group was taught a series of flexibility exercises and encouraged to perform them at home while maintaining their regular lifestyle. Follow-up tests were available for 37 of the 43 people in the control group. Subsequently, 24 control group members opted to complete the exercise program, yielding a total of 65 people studied before and after exercise training.

Because the goal of the study was to examine effects of the exercise program, participants were instructed to maintain body weight. Weight was recorded weekly, and the GCRC dietary staff provided further guidance if weight changed >2%. Only one person in the exercise group discontinued the study because of excessive weight loss.

Procedures. A standard treadmill stress test was performed initially to assure cardiovascular health and was followed on another day with measurement of peak oxygen uptake ($V\mathrm{O}_{2\mathrm{peak}}$) on a bicycle ergometer (36). Expired gases, heart rate, and blood pressure were continuously monitored throughout the tests (36). The posttraining assessment was made within 3 days of the completion of the last training bout.

Insulin sensitivity (S_I) was determined using an intravenous glucose tolerance test (37). Posttraining tests were performed 4 or 5 days after the last exercise session (this variation was unavoidable due to scheduling availability). There were no systematic differences across sex or age in the retesting schedule. Glucose (0.3 g/kg) and insulin (0.03 units/kg) were intravenously injected at 0 and 20 min, respectively. Fifteen blood samples were obtained between 0 and 180 min. S_I was calculated using minimal model analysis software (MINMOD, version 2.0).

Fat and fat-free mass were determined by dual X-ray absorptiometry (Lunar DPX-L, Madison, WI). Abdominal fat was measured using a single-slice (6-mm thickness) computed tomography scan (Imatron C-150, San Francisco, CA) at the level of the L4–L5 intervertebral space. Total and subcutaneous abdominal fat areas were estimated by manual planimetry using custom software (38). Visceral fat area was calculated as the difference between total and subcutaneous fat areas.

Plasma insulin was measured with a two-site immunoenzymatic assay (Access; Beckman Instruments, Chaska, MN). Glucose was measured with a Beckman Glucose Analyzer (Beckman Instruments, Porterville, CA). Nonesterified free fatty acids (NEFAs) were measured using an enzymatic colorimetric assay (NEFA C; Wako Chemicals, Richmond, VA).

A real-time quantitative PCR system (PE Biosystems, Foster City, CA) was used to measure the abundance of selected mRNAs in muscle tissue (35). Transcripts quantified included GLUT4, NRF-1, PGC-1α, TFAM, and two subunits of mitochondrial respiratory chain proteins. The mitochondrial mRNAs were cytochrome c oxidase subunit 4 (COX4), a nuclear gene encoding part of complex IV, and NADH-dehydrogenase subunit 4 (ND4), a mitochondrial-DNA gene encoding part of complex I. RNA was extracted from skeletal muscle of individual subjects by Trizol method (Life Technologies, Gaithersburg, MD), treated with DNase (Life Technologies), and then reverse-transcribed using the TaqMan Reverse Transcription Reagents (PE Biosystems).

The following primers and probes for human genes were used: GLUT4 (designed from GenBank Accession number M20747 sequence) forward primer: CATTGGTATCATCTCT CAGTGGCT, reverse primer: AGCACCGCCA GGACATTG, probe: ACCAGCATGGCCCT TTTCCTTC; NRF-1 (GenBank accession no. XM011548) forward primer: GAGTGATG TCCGCACAGAAGAG, reverse primer: TTATAACAGTTTTTAACTATGGTCCGTAGTG, probe: TGGG TCCATGAAACCCTCTGCTT; PGC-1α (GenBank accession no. AF106698) forward primer: AGATCGCCCTACAGCCGTC, reverse primer: TCTTCAGCCT CTCGTGCTGA, probe: ATTCCTCGTAGCTGTCATACCTGGGC; TFAM (GenBank accession no. M62810) forward primer: TGTGCACCGGCTGTGG, reverse primer: TGGACAACTTGCCAAGACAGAT, probe: AGTCGACTGCGCTCCCC CTT; COX4 (GenBank accession no. XM008055) forward primer: CCTCCTG GAGCAGCCTCTC, reverse primer: TCAGCAAAGCTCTCCTTGA ACTT, probe: TGCGATACAACTCGACTTTCTCATCCAT; ND4 (GenBank accession no. NC 001807) forward primer: CCCCATTCTCCTCCTATCCC, reverse primer: TTTTTTTTTTTT-TTTTTTTTTTTTTTTAAGAG, probe: CAACCCCGACATCATT ACCGGGT. The probes for nuclear genes were designed to span exon boundaries to ensure no amplification of contaminating DNA. Because the mitochondrial genome does not contain introns, the reverse primer for ND4 was designed to target several of the final nucleotides specific to the gene as well as a string of the poly-A tail that is present only in the mRNA. Samples were run in triplicate and quantified by normalizing the target signal for the 28S rRNA signal in each sample.

Activities of two mitochondrial enzymes, citrate synthase and cytochrome c oxidase, were measured in muscle homogenates as previously described (32).

Abundance of GLUT4 protein in muscle was determined by Western blotting. Muscle samples were homogenized in a buffer containing 250 mmol/l sucrose, 20 mmol/l HEPES, and 1 mmol/l EDTA, pH 7.4, containing 1 μg/ml

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TABLE 2
Univariate correlations between age, insulin sensitivity index, and selected variables

Variable	Age		Insulin sensitivity	
	Women	Men	Women	Men
Abdominal fat, total (cm^2)	0.41*	0.38*	−0.62*	−0.67*
Abdominal fat, visceral (cm^2)	0.51*	0.41*	−0.61*	−0.58*
Abdominal fat, subcutaneous (cm^2)	0.28	0.22	−0.56*	−0.57*
Waist circumference (cm)	0.42*	0.39*	−0.59*	−0.43*
Total body fat (%)	0.44*	0.32*	−0.43*	−0.28
Total body FFM (kg)	−0.32*	−0.35*	−0.16	−0.21
BMI (kg/m^2)	0.35*	0.25	−0.43*	−0.55*
Total cholesterol (mmol/l)	0.57*	0.36*	−0.26	−0.36*
Triglycerides (mg/dl)	0.47*	0.27	−0.36*	−0.59*
Insulin (pmol/l)	0.37*	0.02	−0.55*	−0.60*
Glucose (mmol/l)	0.49*	0.42*	−0.25	−0.40*
Nonesterified fatty acids (μmol/l)	0.28	0.38*	0.03	−0.30
$V_{O_{2peak}}$ ($ml \cdot kg^{-1}$ FFM $\cdot min^{-1}$)	−0.84*	−0.82*	0.44*	0.31*
Citrate synthase (units/min)	−0.37*	0.03	0.36*	0.51*
Cytochrome c oxidase (units/min)	−0.46*	−0.36*	0.31	0.37*
COX4 mRNA (arbitrary units)	−0.32*	−0.45*	−0.03	0.23
ND4 mRNA (arbitrary units)	−0.45*	−0.50*	0.18	0.20
GLUT4 mRNA (arbitrary units)	0.29	0.04	0.07	0.29

*$P < 0.025$.

leupeptin, 10 μg/ml aprotinin, and 2 mmol/l phenylmethylsulfonyl fluoride, then centrifuged at 100,000*g* for 60 min. Pellets were resuspended in phosphate buffer containing 1% Triton X-100 and centrifuged at 14,000*g* for 20 min. The resulting supernatants, containing membrane proteins, were separated on 10% polyacrylamide gels (BioRad Laboratories, Hercules, CA). Proteins were transferred to polyvinylidene difluoride membranes and incubated overnight with a primary antibody against GLUT4 (Biogenesis, Brentwood, NH). An enhanced chemiluminescent detection system (Amersham Biosciences, Piscataway, NJ), followed by digital imaging and densitometry (ImageStation 1000; Eastman Kodak Scientific Imaging, Rochester, NY), was used to quantify the relative abundance of GLUT4 in individual samples.

Statistical analysis. Data are reported as means ± SE. Differences between men and women and between pre- and posttesting within groups were analyzed using unpaired and paired *t* tests, respectively. Pearson correlation coefficients were used to measure association among selected variables. Multiple regression analysis was used to determine the relationship of age, body composition, and other variables to insulin sensitivity. $P < 0.05$ was considered statistically significant.

RESULTS

Physical characteristics and body composition. The baseline physical characteristics of the participants are given in Table 1, and univariate correlations between these variables and age are listed in Table 2. Although BMI was similar among age-groups, fat-free mass decreased with age and total body fat and abdominal fat increased with age. Subcutaneous abdominal fat also tended to increase with age, but this change was more variable. Exercise resulted in statistically significant reductions in body weight (0.7%), BMI (0.7%), abdominal fat (5.3%), and waist circumference (1.9%) (Table 3). The loss of abdominal fat occurred equally in the visceral and subcutaneous compartments. Except for a small but significant increase in

TABLE 3
Aerobic capacity, body composition, blood variables, and insulin sensitivity before and after control or exercise training periods

Variable	Control		Exercise	
	Before	After	Before	After
$V_{O_{2peak}}$ ($ml \cdot kg^{-1}$ FFM $\cdot min^{-1}$)	40.7 ± 1.4	40.3 ± 1.2	40.5 ± 1.1	44.3 ± 1.2*
Weight (kg)	77.8 ± 2.0	78.1 ± 2.0	79.2 ± 1.6	78.7 ± 1.6*
Waist circumference (cm)	89.7 ± 1.7	91.9 ± 1.5*	91.9 ± 1.4	90.0 ± 1.5*
BMI (kg/m^2)	25.7 ± 0.3	25.9 ± 0.4	26.6 ± 0.3	26.4 ± 0.3*
FFM (kg)	49.1 ± 1.8	49.3 ± 1.8	50.1 ± 1.4	50.1 ± 1.4
Body fat (%)	31.2 ± 1.3	31.0 ± 1.3	31.4 ± 0.9	31.0 ± 0.9
Abdominal fat, total (cm^2)	299 ± 23	299 ± 22	318 ± 17	299 ± 18*
Abdominal fat, visceral (cm^2)	122 ± 13	121 ± 12	133 ± 11	124 ± 11*
Abdominal fat, subcutaneous (cm^2)	177 ± 13	178 ± 13	185 ± 9	174 ± 10*
Total cholesterol (mmol/l)	5.07 ± 0.17	4.85 ± 0.19	4.90 ± 0.16	4.66 ± 0.12
HDL cholesterol (mmol/l)	1.25 ± 0.12	1.15 ± 0.08	1.17 ± 0.06	1.33 ± 0.11
LDL cholesterol (mmol/l)	3.03 ± 0.15	2.99 ± 0.18	3.00 ± 0.15	2.76 ± 0.13
Triglycerides (mg/dl)	153 ± 13	137 ± 14*	146 ± 11	114 ± 7*
Fasting insulin (pmol/l)	35 ± 3	37 ± 3	38 ± 3	34 ± 2
Fasting glucose (mmol/l)	5.4 ± 0.1	5.3 ± 0.1	5.3 ± 0.1	5.3 ± 0.1
Nonesterified fatty acids (μmol/l)	520 ± 22	512 ± 18	524 ± 15	494 ± 14*
Insulin sensitivity index ($\times 10^{-5} \cdot min^{-1} \cdot pmol^{-1} \cdot l^{-1}$)	5.50 ± 0.53	5.22 ± 0.58	4.88 ± 0.36	6.27 ± 0.60*

Data are means ± SE. *Before-to-after difference within group, $P < 0.025$. None of the before-to-after changes differed with age or sex, except insulin sensitivity.

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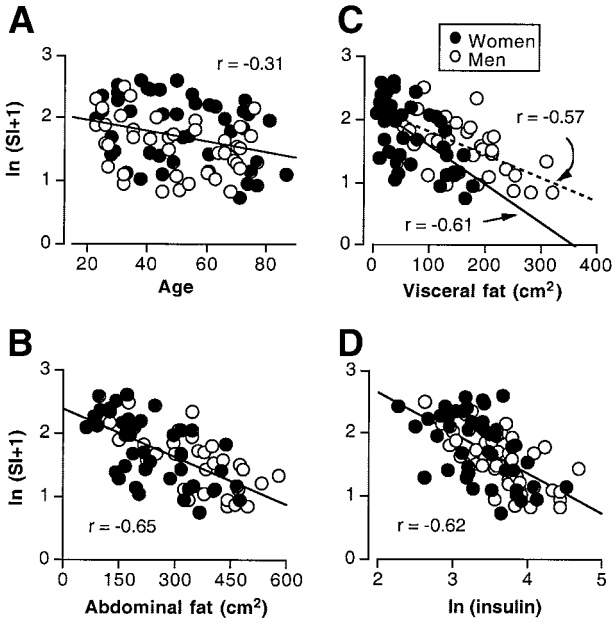

FIG. 1. Relationship between S_I and age, adiposity, and insulin levels. The variance in S_I and insulin was not evenly distributed, so data are shown after log transformation. S_I declined with age (*A*) but was more closely related to total abdominal fat (*B*), visceral fat (*C*), and fasting insulin (*D*) levels. Regression lines are shown for pooled data from men (○) and women (●), except in *C*, where the solid line is for women and the dotted line is for men. $P < 0.01$ for all correlation coefficients shown.

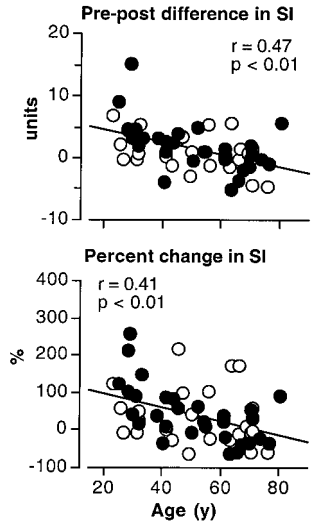

FIG. 2. Relationship between age and the post-exercise change in S_I. The absolute increment in S_I in exercisers was inversely related to age (top panel, regression line for entire group). The relationship was significant ($P < 0.01$) for both women ($r = 0.53$) and men ($r = 0.34$) when considered separately. The percent change in S_I (lower panel, regression line for entire group) was also inversely related to age, but when considered separately, the effect was significant in women ($r = 0.61$, $P < 0.01$) but not in men ($r = 0.16$, $P > 0.05$). S_I units in the top panel are $\times 10^{-5} \cdot \text{min}^{-1} \cdot \text{pmol}^{-1} \cdot \text{l}^{-1}$.

waist size (Table 3), the physical characteristics of the control group did not change during the study.

Aerobic capacity. At baseline, $V\text{O}_{2\text{peak}}$ declined linearly with age from a mean of 50.0 ± 1.0 ml · kg^{-1} FFM · min^{-1} in 20- to 30-year-olds to 19.7 ± 1.1 in those ≥70 years ($r = 0.82$, $P < 0.001$). The rate of decline was 7.5% per decade. There was no change in $V\text{O}_{2\text{peak}}$ in control subjects (Table 3), whereas the exercise program increased $V\text{O}_{2\text{peak}}$ by 9.5% ($P < 0.001$). This response to exercise training was similar in men and women of all ages.

Insulin sensitivity. S_I declined ~8% per decade in both men and women ($P < 0.01$). When plotted against age, however, the residual errors for S_I were not uniform for either men or women. For this reason, linear modeling of S_I was performed after log transformation. The transformed S_I was 16% higher in women ($P < 0.05$) but did not prevent the pooled data from both sexes from being fit by a single line, since the difference was not affected by age (Fig. 1). Univariate correlations between S_I (after log transformation) and other variables are listed in Table 2. S_I was more closely related to abdominal fat and fasting insulin levels than age (Fig. 1). Like S_I, insulin values were also log transformed because of nonuniform distribution.

Multiple regression analyses were used to determine the effects of age, adiposity, and other parameters on S_I at baseline. Men and women were analyzed separately. Using step-wise (step-up) regression, only two variables with significant individual contributions were entered into the model for men, namely total abdominal fat area and triglycerides. This model had a multiple R^2 of 0.57 and an adjusted R^2 of 0.54. When a step-down approach was used for men, total abdominal fat and insulin were retained in the model (multiple $R^2 = 0.55$, adjusted $R^2 = 0.51$). In women, both the step-up and step-down models had a single variable, total abdominal fat area, with a multiple R^2 of 0.39 and adjusted R^2 of 0.37. Combining men and women in the regression analysis yielded a model with total abdominal fat and insulin as predictors (multiple $R^2 = 0.48$, adjusted $R^2 = 0.47$). Age made a significant contribution to these models only if total abdominal fat and/or insulin were intentionally left out (data not shown).

Exercise training increased insulin sensitivity by an average of 26% (Table 3). However, the response to training (absolute change) was inversely related to age in men and women (Fig. 2). When separated into younger (20−39 years), middle-aged (40–59 years), and older (≥60 years) groups, the posttraining S_I increased 72% for younger (5.77 ± 0.73 vs. 9.92 ± 1.36; $P < 0.001$) and 20% for middle-aged (5.42 ± 0.68 vs. 6.52 ± 0.82; $P = 0.11$) and decreased 5% for older (3.90 ± 0.44 vs. 3.71 ± 0.53; $P = 0.42$) people. The response of S_I to training was not related to changes in $V\text{O}_{2\text{peak}}$, body composition, muscle metabolic parameters, or any other variables, besides age. When the S_I values in the exercise group were expressed as a percentage change from baseline, the negative relationship with age remained statistically significant in women but not in men (Fig. 2). This difference between men and women was due to the strong influence of three men over 40 years of age who began the study with low S_I values and had small absolute changes that represented high percentage changes. When considered separately, the percentage change in S_I in exercising young men ($41 \pm 19\%$ for $n = 6$; $P = 0.08$ vs. zero) was less than that of young women ($116 \pm 27\%$ for $n = 9$; $P < 0.01$ vs. zero) but stands in contrast to the middle-aged and older groups who showed no trends toward a positive change in S_I. Thus, the age-dependent increase in S_I after training appears to be present in men and women when considered as either absolute or percentage change.

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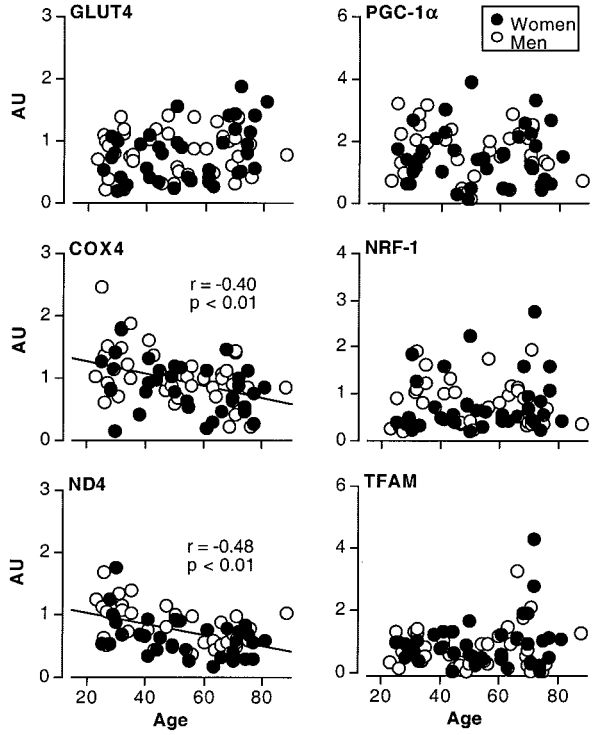

FIG. 3. Effect of age on abundance of mRNA for selected genes in skeletal muscle. Measurements were performed on 78 people during the baseline phase of the study. Regression lines are given for COX4 and ND4, which declined significantly with age. The other genes shown did not change with age. Values are given in arbitrary units (AU) after normalization to 28S rRNA.

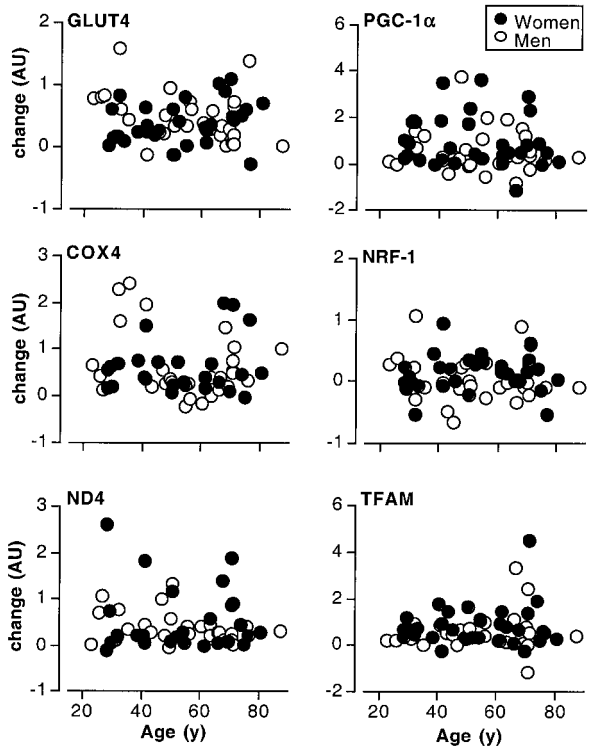

FIG. 4. Effect of the aerobic training program on mRNA abundance of selected genes in skeletal muscle. Exercise caused an increase in the level of each of these mRNAs (averages given in Table 4). This figure demonstrates that the responses to training did not differ with age or sex. Values are given as the absolute difference between the pre- and posttraining measurements, in arbitrary units (AU) after normalization to 28S rRNA.

GLUT4. GLUT4 mRNA levels did not differ with age among the participants (Fig. 3). After the exercise training program, GLUT4 mRNA levels were elevated by 52% (Table 3), and no effect of either age or sex on the response to exercise was evident. GLUT4 protein levels were measured on a subset of younger ($n = 9$) and older ($n = 11$) persons because of limited availability of muscle samples. GLUT4 protein abundance (in arbitrary units) was not significantly different between the younger (5.71 ± 0.41 AU) and older (4.67 ± 0.49 AU) group at baseline. In younger and older exercisers, protein levels were increased 20–40% to 6.83 ± 0.72 and 6.64 ± 0.62 AU, respectively ($P < 0.02$), with no difference between age groups.

Mitochondrial enzyme activities and gene transcripts. At baseline, activity of COX declined with age (5% per decade) (Table 2). Citrate synthase activity declined with age in women (4% per decade) (Table 2) but not in men. The exercise program increased COX and citrate synthase activities by 87 and 46%, respectively (Table 3). Enzyme changes with training were not related to either age or sex.

Transcript levels of COX4 and ND4 followed similar patterns to one another, as they both declined significantly with age (Fig. 3) and increased 65–67% with aerobic exercise training (Table 4). These responses to training were not affected by either age or sex. The abundance of mRNAs encoding PCG-1α, NRF-1, and TFAM was unrelated to age at baseline (Fig. 4). Exercise training in-

TABLE 4
Values for muscle variables before and after control or exercise training periods

Variable	Control		Exercise	
	Before	After	Before	After
Citrate synthase activity	24.0 ± 1.2	25.1 ± 1.2	24.1 ± 0.8	35.1 ± 1.3*
Cytochrome c oxidase activity	6.3 ± 0.6	7.3 ± 0.8	7.1 ± 0.5	13.3 ± 0.7*
COX4 mRNA	1.00 ± 0.08	1.02 ± 0.09	0.92 ± 0.06	1.54 ± 0.13*
ND4 mRNA	0.76 ± 0.06	0.77 ± 0.06	0.73 ± 0.04	1.19 ± 0.09*
PCG-1 mRNA	1.66 ± 0.15	1.49 ± 0.13	1.48 ± 0.10	2.29 ± 0.15*
NRF-1 mRNA	0.83 ± 0.10	0.80 ± 0.10	0.71 ± 0.06	0.82 ± 0.07*
TFAM mRNA	0.91 ± 0.15	0.99 ± 0.14	0.81 ± 0.07	1.50 ± 0.13*
GLUT4 mRNA	0.87 ± 0.07	0.92 ± 0.08	0.82 ± 0.06	1.25 ± 0.07*

Data are means ± SE. Enzyme activities are given as $\mu mol \cdot min^{-1} \cdot g^{-1}$ tissue; mRNA levels, as arbitrary units after normalization to 28S rRNA. *Before-to-after difference within group, $P < 0.001$ for all variables except NRF-1, which was $P < 0.02$. None of the changes in the exercise group had a significant interaction with age or sex.

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creased PGC-1α, NRF-1, and TFAM transcript levels by 55, 15, and 85%, respectively, but there was no interaction between age and the training response (Table 4). PGC-1 mRNA levels at baseline were positively associated ($P < 0.01$) with abundance of NRF-1 ($r = 0.67$), TFAM ($r = 0.53$), GLUT4 ($r = 0.46$), and COX4 ($r = 0.40$) mRNA. The change in PGC-1α mRNA after training was associated with the change in NRF-1 ($r = 0.40$, $P < 0.01$), but not with the changes in TFAM, GLUT4, or COX4.

Plasma lipids, glucose, and insulin. Total cholesterol increased from 4.24 ± 0.18 mmol/l in 20- to 30-year-olds to 5.54 ± 0.27 in people over 70 years ($P < 0.025$), whereas HDL and LDL cholesterol levels did not change with age (Table 2). Cholesterol levels were not significantly altered by exercise training (Table 3). Triglyceride (134 ± 15 mg/dl, 20–30 years; 160 ± 15, ≥70 years) and nonesterified fatty acid (485 ± 34 μmol/l, 20–30 years; 587 ± 27, ≥70 years) levels both increased with age (Table 2). Exercise training lowered fasting plasma triglycerides 23% and nonesterified fatty acids 6% (Table 3). Age and sex did not affect these responses.

Fasting glucose levels increased 2% per decade in men and women, whereas insulin increased 13% per decade in women but did not change with age in men (Table 2). Fasting glucose and insulin were positively related ($P < 0.01$) to abdominal fat ($r = 0.56$ and 0.65, respectively). Men had higher ($P < 0.01$) plasma glucose (5%) and insulin (16%) than women. Overall, exercise training did not alter fasting glucose or insulin concentrations (Table 3). When separated into three age-groups, however, fasting insulin levels were significantly reduced from pre- to postexercise in middle-aged people (age 40–59 years, 36 ± 3 vs. 31 ± 3 pmol/l; $P = 0.020$) and showed a tendency to decline in older people (≥60 years, 44 ± 5 vs. 35 ± 3 pmol/l; $P = 0.098$). Fasting insulin did not change in younger exercisers (20–39 years old, 32 ± 5 vs. 37 ± 7 pmol/l; $P = 0.40$). Fasting glucose levels were unchanged in any age-group.

DISCUSSION

This study examined the effect of a 4-month moderate-intensity aerobic exercise program on insulin sensitivity and muscle mitochondrial biogenesis in people between the ages of 22 and 87 years. In agreement with previous reports, insulin sensitivity declined with age (5–8) but improved with exercise training (9–14). A key finding of the current study is that the increment in insulin sensitivity due to exercise training was present in younger people but not in middle-age and older groups. Several potential factors that might explain the age-dependent change in insulin action with exercise were examined. Abdominal adiposity (which was the best correlate with insulin sensitivity at baseline) and plasma triglycerides were decreased by exercise. In skeletal muscle, glucose transporter mRNA and protein levels were increased, and mitochondrial biogenesis was evident from increased activity of mitochondrial enzymes and higher levels of mitochondrial protein mRNAs. These changes occurred in men and women across the age span. Thus, insulin sensitivity was the only variable that demonstrated an age-dependent response to exercise training.

Insulin-mediated glucose transport in muscle depends on the content and function of GLUT4 (21). Work by Houmard et al. (6) suggested that the age-related decline in insulin action might be due in part to a lower abundance of GLUT4 protein in muscle. Subsequently, it was reported that short-term vigorous training elevated GLUT4 protein content and insulin sensitivity in young and old people (12). In the current study, we found that muscle GLUT4 mRNA and protein levels did not change with age in previously untrained people. Exercise training resulted in increased muscle content of GLUT4 mRNA and protein, but this increment did not differ with age. Thus, in the present study, muscle GLUT4 abundance does not explain the age-related decline in insulin sensitivity or the age-dependent response to exercise training. One possibility is that despite normal GLUT4 content in older muscle, the insulin-mediated trafficking of GLUT4 to the sarcolemma or its inherent function is somehow impaired (20,21). At present, the interaction of age and exercise status on GLUT4 trafficking and transport function is unknown. This is an important question that should be addressed in future work.

The activity of oxidative enzymes is reduced in skeletal muscle from older people (32,34,39) and can contribute to reductions in $V_{O_{2peak}}$ and the ability to sustain muscular activity. In turn, this could potentially contribute to insulin resistance by limiting the ability to metabolize glucose. There is evidence that people with type 2 diabetes or insulin resistance have lower activity of oxidative enzymes and reduced aerobic capacity (24,25,40). In the present study, $V_{O_{2peak}}$ and activity of mitochondrial enzymes were positively related to S_I at baseline. Despite this relationship, muscle mitochondrial variables did not contribute significantly to the multivariate model for S_I after the inclusion of abdominal fat. We found that aerobic exercise training increased the glucose transport capacity (higher GLUT4) and mitochondrial content of skeletal muscle as previously reported (22,23). However, these changes were not related to the change in S_I. These findings suggest that insulin action and mitochondrial biogenesis may respond to common regulatory events, such as exercise, but that the two pathways are not directly related.

We have considered other factors that may explain the age-dependent exercise response in insulin sensitivity. First, compared to the current study, training programs that improved insulin sensitivity in older people typically had more intense or frequent sessions (12,13) or were longer (6–12 months) (9,11,13). In one study, 63-year-old people engaged in low-intensity exercise (e.g., walking) showed no change in insulin action after 6 months, but improved after an additional 6 months of more intensive exercise (9). Thus, older people may require more intensive or longer exercise training programs to improve S_I, whereas other factors, such as mitochondrial function, are more readily altered. A second possibility is that the effect of exercise on S_I response is more rapidly lost in older people. Timing of the posttraining measurements of S_I could be important. In most studies, S_I was measured 14−17 h after the last exercise session (9,11,12), whereas we waited 96–120 h to examine chronic training adaptations instead of acute exercise effects. Although there was some variation in the timing of our follow-up tests (≤24 h), there was no systematic difference in the time interval

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across age or sex, nor was there an effect on the magnitude in S_I improvement. In well-trained athletes, S_I declines within 6–14 days if regular exercise is not maintained (14,41,42). Future work will need to examine how S_I changes in the first 7 days after completing a training program with comparisons made between younger and older people. A third explanation is that inherent aging processes may blunt the adaptability of S_I even if regular vigorous exercise is performed. It was recently shown that S_I is lower in both older sedentary and endurance-trained people versus younger people with similar respective exercise status (43). Further, as we and others have found (7,8,44), the age effect on S_I disappeared in sedentary subjects after controlling for body fatness (43). In contrast, after controlling for body fat, S_I was still 33% lower in older athletes (43). Insulin sensitivity in the older runners was equal to or greater than that in sedentary groups, indicating a clear benefit of maintaining a long-term vigorous exercise training program (43). Nevertheless, the lower S_I in older versus younger runners suggests that the capacity to improve insulin action with exercise may be somehow limited in older people.

Exercise training resulted in an average reduction in body weight of 0.6 kg, which appears to have come mainly from abdominal fat stores. This reduction was similar in visceral and subcutaneous abdominal fat depots and did not vary with age. Thus, despite the close correlation between abdominal fat and S_I at baseline, the magnitude of the improvement in S_I after exercise training was not related to the amount of fat lost. In overweight men, exercise training and weight loss are attributed with distinct effects on glucose metabolism that are additive when combined (45). We encouraged participants to maintain their diet and body weight in this study, so it is possible that a weight-loss component would have resulted in greater gains in insulin sensitivity in the exercise group (45,46).

Fasting insulin and glucose showed little or no change with exercise training and were therefore unrelated to changes in S_I. Exercise-induced improvements in S_I in the absence of a change in fasting insulin or glucose have been noted before (12,47). When reductions in fasting insulin occur in response to exercise training, a common cofactor is weight loss, specifically fat loss (9,11,13,45). Fasting insulin levels are positively associated with body fat (r = 0.65 in the present study at baseline). The exercise group had a 5% reduction in abdominal fat, but a larger decrease may be required to substantially affect fasting insulin levels.

At baseline we found that S_I decreased with age, consistent with other reports (5–8). This decline appears to result from an increase in abdominal fat rather than age per se. Earlier reports found a relationship between S_I and anthropometric measures like waist circumference or BMI (7,8,44). In the current study, S_I was more closely related to visceral than subcutaneous fat, but S_I was most closely related to total abdominal fat area, in agreement with the findings of Goodpaster et al. (48). A consensus has not been reached on whether visceral or subcutaneous fat regions are more important for conferring insulin resistance (48–50).

The decline with age in activity of mitochondrial enzymes indicates that muscle oxidative capacity is reduced (32,34,39). Previous work suggested that mitochondrial functional decline might be related to lower synthesis rate of mitochondrial proteins (32). The current results now indicate that protein synthesis in older muscle may be limited by template availability, since mRNA levels for both nuclear-encoded (COX4) and mitochondrial-encoded (ND4) respiratory chain proteins decreased with age. We tested whether these changes were related to parallel reductions in transcription factors that regulate mitochondrial genes upstream. PGC-1α has generated interest because it acts with transcription factors like NRF-1 to control expression of genes of oxidative metabolism, including TFAM (51,52). TFAM is the signal through which the nucleus regulates mitochondrial DNA transcription and replication (53). The mRNA level of PGC-1α was positively associated at baseline with that of GLUT4, NRF-1, and TFAM, supporting the evidence that PGC-1α is a key regulator of energy-utilizing pathways. However, PGC-1α, NRF-1, and TFAM transcript abundance did not change with age. This implies that the reduction in COX4 and ND4 mRNA with age must be regulated at other control points such as mRNA stability or transcription factor activity, or perhaps by other transcription factors. Oxidative damage to DNA has been shown to increase with age (54) and could limit the expression of specific mitochondrial genes in the presence of normal levels of transcription factors. We are aware of only one other study that has examined age effects on mitochondrial-related transcription factors in human muscle (55). In contrast to our findings, mRNA levels of TFAM and NRF-1 were reported to be higher in a group of elderly (71–88 years) than in younger (21–33 years) people (55). However, the muscle samples in that study were obtained from only four to seven people per group who were undergoing orthopedic surgery. The clinical state, anesthesia, or any treatments could modulate the mRNA level of these transcription factors, and the data may not be applicable to healthy populations.

The exercise training program resulted in an enhancement of mitochondrial biogenesis, as shown by several markers. There were no interactions between age and the training response of the mitochondrial measurements. Improvements in $V\mathrm{O}_{2\mathrm{peak}}$ and oxidative enzymes in response to aerobic exercise have been previously shown in older people (56,57). Earlier studies also showed that vigorous training results in higher muscle mRNA content of TFAM and other mitochondrial genes in young men (58,59). The present study is the first to show that aerobic training stimulates expression of mitochondrial genes and transcription factors in muscle from older and younger people. The potency of the training effect on muscle oxidative pathways is shown by the fact that all of the changes in muscle that were measured were clearly evident 5–6 days after the last training session. Collectively, these findings demonstrate that despite age-related functional decline, skeletal muscle capacity for mitochondrial biogenesis remains high in older muscle when faced with the metabolic demands of regular exercise. Exercise-induced increases in muscle oxidative enzymes are consistent with higher ATP production capacity (56) and may lead to greater ability to utilize glucose. However, the

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present data demonstrate that changes in muscle mitochondrial function and insulin-mediated glucose disposal are not closely related.

In conclusion, 4 months of moderate-intensity aerobic exercise performed by previously sedentary men and women improved insulin sensitivity in young people but not in middle-aged and older groups. This unique finding could not be explained by loss of body fat or increased expression of glucose transporter protein in skeletal muscle, since exercise training had an equivalent effect on these parameters in people of all ages. Muscle mitochondrial enzyme activity declined with age and improved with exercise training regardless of age. The exercise-induced increase in muscle oxidative capacity was closely related to the availability of the mRNA abundance of mitochondrial proteins. These findings suggest that the ability of aerobic exercise to enhance muscle mitochondrial function is not age-limited, whereas improvement in insulin sensitivity is impaired in older people. This observation will need to be considered in the design of exercise programs aimed at preventing or delaying diabetes in older people.

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