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Insulin-resistant muscle is exercise resistant: evidence for reduced response of nuclear-encoded mitochondrial genes to exercise

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De Filippis E, Alvarez G, Berria R, Cusi K, Everman S, Meyer C, Mandarino LJ. Insulin-resistant muscle is exercise resistant: evidence for reduced response of nuclear-encoded mitochondrial genes to exercise. *Am J Physiol Endocrinol Metab* 294: E607–E614, 2008. First published January 8, 2007; doi:10.1152/ajpendo.00729.2007.—Mitochondrial dysfunction, associated with insulin resistance, is characterized by low expression of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) and nuclear-encoded mitochondrial genes. This deficit could be due to decreased physical activity or a decreased response of gene expression to exercise. The objective of this study was to investigate whether a bout of exercise induces the same increase in nuclear-encoded mitochondrial gene expression in insulin-sensitive and insulin-resistant subjects matched for exercise capacity. Seven lean and nine obese subjects took part. Insulin sensitivity was assessed by an 80 $mU \cdot m^{-2} \cdot min^{-1}$ euglycemic clamp. Subjects were matched for aerobic capacity and underwent a single bout of exercise at 70 and 90% of maximum heart rate with muscle biopsies at 30 and 300 min postexercise. Quantitative RT-PCR and immunoblot analyses were used to determine the effect of exercise on gene expression and protein abundance and phosphorylation. In the postexercise period, lean subjects immediately increased PGC-1α mRNA level (reaching an eightfold increase by 300 min postexercise) and protein abundance and AMP-dependent protein kinase phosphorylation. Activation of PGC-1α was followed by increase of nuclear respiratory factor-1 and cytochrome *c* oxidase (subunit VIc). However, in insulin-resistant subjects, there was a delayed and reduced response in PGC-1α mRNA and protein, and phosphorylation of AMP-dependent protein kinase was transient. None of the genes downstream of PGC-1α was increased after exercise in insulin resistance. Insulin-resistant subjects have a reduced response of nuclear-encoded mitochondrial genes to exercise, and this could contribute to the origin and maintenance of mitochondrial dysfunction.

insulin resistance; mitochondrial function; exercise; peroxisome proliferator-activated receptor-γ coactivator-1α; AMP-dependent protein kinase

INSULIN RESISTANCE IN SKELETAL MUSCLE is a characteristic of obesity and type 2 diabetes mellitus (8). Recently, the concept that mitochondrial dysfunction may, in part, explain insulin resistance has gained support (16, 18, 21). In skeletal muscle of insulin-resistant subjects, ATP turnover is reduced (22), and this reduced energy demand may lead to an accumulation of intramyocellular lipids, which, in turn, could inhibit insulin signaling (17). A number of studies have shown a reduction in a cluster of oxidative genes under the control of peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1) (16, 21). PGC-1α is expressed in tissues with high-oxidative capacity, such as heart, slow-twitch skeletal muscle, and brown adipose tissue, and is considered to be a critical regulator of mitochondrial biogenesis and functional capacity through an increase in expression of nuclear-encoded mitochondrial genes (32). In particular, PGC-1α coactivates nuclear respiratory factor-1 (NRF-1) and -2 (NRF-2). NRFs regulate expression of mitochondrial transcription factor A, a nuclear-encoded transcription factor essential for replication, maintenance, and transcription of mitochondrial DNA (26a). NRF-1 and -2 also control the expression of nuclear genes encoding respiratory chain subunits and other proteins involved in mitochondrial function (26a).

Mootha et. al. (16) showed that lower expression in this cluster of genes was correlated with reduced whole body aerobic capacity in patients with type 2 diabetes mellitus. Physical activity ameliorates insulin resistance and has a beneficial role in the prevention of type 2 diabetes (14), but the mechanism involved in this positive effect is not fully understood. Regular physical activity increases skeletal muscle mitochondrial mass (11), and, since PGC-1 can drive mitochondrial biogenesis, it is likely that PGC-1α may be responsible for this effect of exercise. Studies in rodents have shown an increase in PGC-1 mRNA between 5- and 7- to 10-fold following a single bout of exercise (2), lending weight to this notion. Moreover, AMP-dependent protein kinase (AMPK) is activated by a low ATP-to-AMP ratio in response to exercise in both animal models and humans (4, 9, 30, 31), and its activation increases glucose and fatty acid oxidation. More recently, AMPK activation, in response to either a single exercise bout (29) or training (28), has been linked to further activation of PGC-1α as a potential mechanism responsible for the training-induced increase in mitochondrial function and biogenesis.

The association of decreased expression of nuclear-encoded mitochondrial genes and reduced mitochondrial dysfunction with insulin resistance might be explained in two ways. First, since exercise is well known to increase the number and activity of mitochondria (11), decreased mitochondrial function in insulin resistance could be merely due to decreased physical activity. Alternatively, insulin-resistant muscle could be less responsive to the ability of exercise to increase expression of nuclear-encoded mitochondrial genes, leading to the relationship between decreased mitochondrial function and insulin resistance. To differentiate between these possibilities, we assessed the ability of a single bout of exercise to increase

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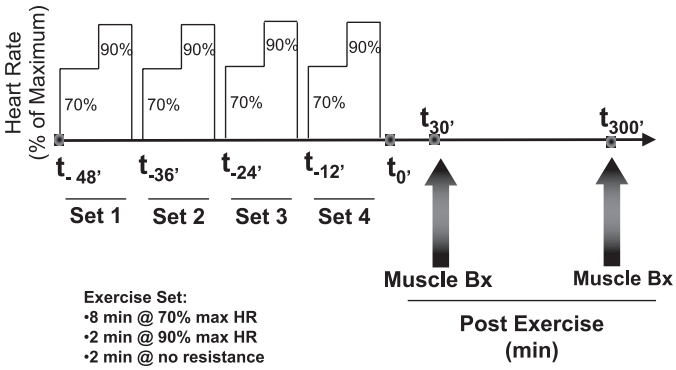

Fig. 1. Design of the exercise session. The exercise session was organized into 4 sets; exercise was carried out on a stationary recumbent cycle. In each set, subjects cycled for 8 min at moderate intensity [70% of their individual maximum heart rate (HR)], then for 2 min at high intensity (90% of their individual maximum HR), followed by 2 min with no resistance. This set was repeated 4 times. Throughout the exercise session, HR was monitored using a 3-lead ECG and recorded every 2 min. Biopsies (Bx) of the vastus lateralis muscle were performed 30 min ($t_{30'}$) and 5 h ($t_{300'}$) after completion of the 4 sets of exercise.

expression of PGC-1 and nuclear-encoded mitochondrial genes in lean, insulin-sensitive and obese, insulin-resistant subjects, who were matched for exercise capacity and did not engage in regular physical activity.

METHODS

Subjects. Seven lean control and nine obese nondiabetic subjects were recruited for this study. The study was approved by the Institutional Review Boards of the University of Texas Health Science Center at San Antonio, the Carl T. Hayden Veterans Administration Health Center, and Arizona State University. All studies were conducted at either the General Clinical Research Center of the University of Texas Health Science Center at San Antonio or at the Carl Hayden Veterans Affairs (VA) Hospital Diabetes Research Unit. Informed, written consent was obtained from all subjects. None of the volunteers engaged in regular exercise, nor did they change their total body weight for at least 6 mo before participating in this study. As part of the initial screening visit, a medical history, physical examination, 12-lead electrocardiogram, and a complete chemistry panel were obtained. A 75-g oral glucose tolerance test was performed using American Diabetes Association criteria to exclude nondiabetic subjects with impaired glucose tolerance. No subjects were taking any medication known to affect glucose metabolism.

Peak aerobic activity. Peak aerobic activity ($\dot{V}O_{2peak}$) was determined using an electrically braked cycle ergometer and a Sensormedics model V29 Metabolic Measurement System (Sensormedics, Savi Park, CA), as previously described (6). Briefly, exercise was started at a workload of 40 W and increased by 10 W/min until perceived exhaustion or a respiratory quotient of 1.10 was reached. Heart rate and rhythm were monitored using a 12-lead electrocardiogram. Lean and obese subjects participating in the study were selected so as to be matched for exercise capacity ($\dot{V}O_{2peak}$ and maximal work rate).

Euglycemic hyperinsulinemic clamp with basal muscle biopsy. Subjects reported to the General Clinical Research Center at the University of Texas Health Science Center at San Antonio or the Diabetes Research Unit at the Carl T. Hayden VA Medical Center in Phoenix, AZ, after a 10-h overnight fast. To determine insulin sensitivity, subjects underwent a euglycemic hyperinsulinemic clamp, as previously described (3a). At *time −120 min*, a primed (25 μCi), continuous (0.25 μCi/min) infusion of [3-^{3}H]glucose was started via a catheter placed into an antecubital vein and continued throughout the study. A second catheter was placed in retrograde fashion in a vein on the back of the hand, which was then placed in a heated box (60°C). Baseline arterialized venous blood samples for determining plasma [3-^{3}H]glucose radioactivity and plasma glucose, free fatty acid, and insulin concentrations were drawn at −30, −20, −10, −5, and 0 min. At *time 0 min*, a primed, continuous infusion of regular human insulin (Novolin; Novo Nordisk, Princeton, NJ) was started at a rate of 80 $mU \cdot m^{-2} \cdot min^{-1}$ and was continued for 120 min. Plasma glucose was measured with a glucose analyzer (Beckman Instruments, Fullerton, CA) at ~5-min intervals throughout the euglycemic clamp, and a variable infusion of 20% glucose was used to maintain euglycemia. Sixty minutes before the insulin infusion was started, a percutaneous biopsy of the vastus lateralis muscle was obtained using a Bergstrom cannula. This biopsy served as the basal, preexercise muscle specimen taken under resting, unstimulated conditions. Muscle biopsy specimens (50–200 mg) were immediately blotted free of blood, frozen, and stored in liquid nitrogen until use.

Exercise bout with muscle biopsies. All subjects also underwent an exercise test consisting of a single bout of aerobic exercise. The exercise bout was conducted on a separate day after determination of $\dot{V}O_{2peak}$ and at least 1 wk either before or after the euglycemic hyperinsulinemic clamp. Subjects reported to the General Clinical Research Center or Diabetes Research Unit at about 7 AM after fasting overnight. Upon arrival, vital signs were collected, and an antecubital catheter was placed. Subjects were asked to exercise on a recumbent cycle (Schwinn 205P) for a total of 48 min, not including warm-up. The design of the exercise bout is shown in Fig. 1. Subjects warmed up by pedaling for ~5 min with no resistance. When the subject felt comfortable, resistance was increased until the subject's heart rate reached 70% of maximum heart rate measured during the

Table 1. *Primer sequences for quantitative RT-PCR*

Gene	Accession Number	Forward Primer Sequence (5′→3′)	Reverse Primer Sequence (3′→5′)
β-Actin	NM_001101.2	AAACTGGAACGGTGAAGGTG	AGAGAAGTGGGGTGGCTTTT
PGC-1α	NM_013261	TTTCCTTTTGCCATGGAATC	GAAAGAACCGCTGAACAAGC
NRF-1	NM_005011.2	TGACATTGGAACAGTGACAT	AATGCAGTTTCTTCACCAAT
NRF-2	U_13048	AAATTGAGATTGATGGAACAGAGAA	TATGGCCTGGCTTACACATTCA
cytochrome *c* oxidase subunit VIc	NM_004374	AAGGACGTTGGTGTTGAGGT	TTTCTTTGATCAGCCACACG
cytochrome *c* oxidase subunit VIIc	NM_001867	GTTTGTACTTTGGATCTGCATT	TGGCATATGAGTTCTAGTTTGA
AMPK-α_1	NM_006251	ATGCTGAGGCTCAAGGAAAA	GGAAGCATTTGGCTGTGACT
AMPK-α_2	NM_006252	ACCAGCTTGCAGTGGCTTAT	CAGTGCATCCAATGGACATC
LKB1	NM_000455	GGCCTCCATGCACTTTATGT	CCACAGCTCAAATCCACCTT
STRAD	BK_001542	CAAACCTGGAAGAGCTGGAG	AAAAGCCTTGGAGCAGTGAA
MO25	AF_113536	CAGTCCAGGTTGGAGATCGT	ACTTGCCACACTGCACTCAG

Primer sequences of genes analyzed using quantitative RT-PCR are as described in METHODS. PGC-1α, peroxisome proliferative-activated receptor-γ coactivator-1α; NRF, nuclear respiratory factor; AMPK, AMP kinase; LKB1, serine/threonine kinase like; STRAD, Ste20-related adaptor protein-α; MO25, mouse protein 25-α.

$\dot{V}O_{2peak}$ test; the resistance was then kept stable for 8 min. For the next 2 min, resistance was increased so that the subject's heart rate reached 90% of maximum heart rate. Resistance then was reduced to zero for a 2-min rest interval, completing the first set of exercise. This scheme was repeated for a total of four times. Immediately after completing the fourth set, the subject was moved to a bed where a biopsy of the vastus lateralis muscle was performed within 30 min after completion of the exercise bout (~1.5 h after the start of exercise). A second muscle biopsy followed after 5 h of bed rest, at ~3 PM.

Muscle processing. Muscle samples were homogenized, as previously described (19). Muscle samples were weighed while still frozen and were homogenized in ice-cold lysis buffer (1:10 wt/vol) containing 20 mmol/l Tris·HCl (pH 7.4), 1% Triton X-100, 50 mmol/l NaCl, 250 mmol/l sucrose, 50 mmol/l NaF, 5 mmol/l sodium pyrophosphate, 2 mmol/l dithiothreitol, 4 mg/l leupeptin, 50 mg/l trypsin inhibitor, 0.1 mmol/l benzamidine, and 0.5 mmol/l phenylmethylsulphonyl fluoride. The homogenate was centrifuged at 14,000 *g* for 30 min at 4°C. Homogenates were incubated on ice for 30 min and then centrifuged at 15,000 *g* for 20 min at 4°C. The supernatants were collected, and protein concentrations were measured by the Lowry method. Supernatants were stored at −80°C until use.

SDS-PAGE and immunoblotting. Immunoblot analysis using 50-μg muscle protein was carried out as described (5). Briefly, protein lysates were separated by 7.5% SDS-PAGE and transferred to nitrocellulose membranes. After blocking in Tris-buffered saline with 5% nonfat dry milk, the membranes were incubated overnight at 4°C with antibodies against PGC-1α (Cayman, Ann Arbor, MI), phospho-AMPK-α (Thr^{172}), AMPK-α_1, and AMPK-α_2 (Cell Signaling Technology, Danvers, MA), following the manufacturer's recommendations (28). The blots were then quantified by densitometry (VersaDoc imaging system, model 5000; Bio-Rad, Hercules, CA).

Quantitative RT-PCR. Total RNA was extracted from 30–50 mg of muscle using the acid guanidinium thiocyanate-phenol-chloroform extraction with modification (RNA STAT-60; TEL-TEST, Friendswood, TX). Oligo(dT) single-stranded cDNA was synthesized using iScript cDNA synthesis kit (Bio-Rad). Forward and reverse primers complementary to the human genes of interests (Table 1) were designed using the web-based software Primer 3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and synthesized by IDT Integrated (DNA Technologies, Coralville, IA). RT-PCR was performed using the MyiQ Single-Color RT-PCR detection system (Bio-Rad, Coralville, IA), using the iQ SYBRgreen Supermix reagents (Bio-Rad, Hercules, CA) added to 160 ng of cDNA previously synthesized. To determine the efficiencies of each primer pair, a standard curve was generated by serial dilution of an RNA sample taken from a healthy subject. Each sample was run in duplicate, and the mean value of the duplicate was used to calculate the mRNA expression of the gene of interest and an endogenous control. The quantity of the gene of interest in each sample was normalized to that of β-actin RNA using the comparative ($2^{-\Delta\Delta CT}$) method (15). Statistical comparisons were done using paired *t*-tests.

Analytical determinations. Plasma insulin concentrations were measured by radioimmunoassay (Diagnostic Product, Los Angeles, CA). Tritiated glucose-specific activity was determined using barium hydroxide/zinc sulfate deproteinization of plasma samples, and rates of glucose appearance and disappearance were calculated using steady-state or non-steady-state equations, as appropriate (7). Plasma free fatty acid concentrations were determined by an enzymatic, calorimetric quantification method (Wako, Nuess, Germany).

Statistical analysis. Data are presented as means ± SE. Data were compared using repeated-measures analysis of variance, followed by one- or two-tailed Student's paired *t*-tests, as appropriate. Statistical tests were performed using StatView version 5.0 (SAS Institute, Cary, NC). The significance level was set at 0.05. Analysis of covariance was used to assess any effects of age on glucose metabolism or gene expression and protein abundance changes in response to exercise. To illustrate the variability in response to exercise, box-whisker plots were used. These plots show the median, mean, and 5th and 95th percentile, and data range from lowest to highest value, as well as any skewness in the data.

Table 2. *Characteristics of subjects*

	Lean	Obese
Sex (male/female)	3/3	6/3
Age, yr	33±4	48±3*
BMI, kg/m^2	25±1	32±2*
BW, kg	69±5.5	95±8*
FM, kg	19±2	32±3.5*
FFA, μmol/l	550±0.2	583±0.05
FPG, mmol/l	5.2±0.08	5.4±0.18
FPI, pmol/l	73.6±6.2	130±2.8
Hb A_{1c}, %	5.3±0.2	5.5±0.1
Total cholesterol, mmol/l	4.3±0.22	4.4±0.2
Triglycerides, mmol/l	0.9±0.1	1.4±0.4
HDL cholesterol, mmol/l	1.2±0.1	1.2±0.05
LDL cholesterol, mg/dl	2.7±0.3	2.6±0.1
SBP, mmHg	117±4	126±6
DBP, mmHg	70±3	80±3
Resting heart rate, beats/min	65±5.5	72±3

Subject characteristics are baseline values expressed as means ± SE. BMI, body mass index; BW, body weight; FM, fat mass; FFA, free fatty acid; FPG, fasting plasma glucose; FPI, fasting plasma insulin; Hb A_{1c}, glycosylated hemoglobin; SBP, systolic blood pressure; DBP, diastolic blood pressure. *$P < 0.01$ vs. lean controls.

Table 3. *Exercise characteristics*

	Lean	Obese
Maximum HR, beats/min	170±12	168±6
70% Maximum HR, beats/min	119±8	118±4
90% Maximum HR, beats/min	153±11	152±5
$\dot{V}O_{2peak}$, ml·kg FFM^{-1}·min^{-1}	35.4±1.0	35.0±3.4
Maximum workload, W	149±13	156±9

Values are means ± SE. Peak measurements are maximum values obtained during peak aerobic activity ($\dot{V}O_{2peak}$) test. Heart rate (HR) peak is the mean heart rate during the last minute of exercise during the $\dot{V}O_{2peak}$ test. The maximum workload represents the highest workload reached and sustained, corresponding to the maximum HR and $\dot{V}O_{2peak}$ observed during $\dot{V}O_{2peak}$ test. FFM, fat-free mass.

RESULTS

Subject characteristics and insulin sensitivity. Clinical characteristics of the subjects are shown in Table 2. The obese subjects had a greater body mass index (BMI) and fat mass ($P < 0.01$) and were somewhat older (48 ± 3 vs. 33 ± 4 yr; $P < 0.01$) than the lean group. As expected, all of the other

Table 4. *Rates of glucose metabolism during an 80 mU/m^2 euglycemic hyperinsulinemic clamp*

	Lean	Obese
Glucose production		
Basal	2.6±0.5	2.8±0.4
Insulin	0	0
Glucose disposal		
Basal	2.6±0.5	2.8±0.4
Insulin	9.7±0.7	7.0±0.7*

Values are means ± SE in ml·kg FFM^{-1}·min^{-1}. Endogenous glucose production was calculated during the euglycemic hyperinsulinemic clamp, as described in METHODS. *$P < 0.05$ vs. lean controls.

Table 5. *Expression of mRNA under basal conditions*

Gene	Lean	Obese
PGC-1α	4.14±0.28	3.87±0.22
NRF-1	5.74±0.78	5.80±0.24
NRF-2	5.14±0.51	5.11±0.20
cytochrome *c* oxidase (subunit VIc)	−0.51±0.25	−1.11±0.17
cytochrome *c* oxidase (subunit VIIc)	−3.46±0.48	−3.03±0.21
AMPK-α_1	2.40±0.45	2.92±0.54
AMPK-α_2	0.07±0.29	1.23±0.34*
LKB1	4.66±0.63	4.53±0.61
MO25	0.16±0.35	0.19±0.26
STRAD	8.79±0.80	9.09±0.45

Values are presented as means ± SE of change in threshold cycle values compared with β-actin control mRNA values for each individual. β-Actin threshold cycle values were 23.3 ± 0.1 and 23.7 ± 0.1 in lean and obese subjects, respectively (P = not significant). Basal muscle biopsies samples were obtained during the euglycemic, hyperinsulinemic clamp as described in the METHODS section. Quantification of the above genes was assessed by quantitative RT-PCR using 160 ng cDNA, as described in METHODS. *$P < 0.05$ vs. lean controls by *t*-test.

clinical parameters, such as Hb A_{1c}, fasting plasma glucose, lipid profile, and blood pressure, were comparable between the lean and obese individuals. Expressed relative to lean body mass, the lean and obese groups were matched for $\dot{V}O_{2peak}$ (35.0 ± 1.2 vs. 35.0 ± 3.4 ml·kg fat-free mass^{-1}·min^{-1}) and maximum heart rate (170 ± 12 vs. 168 ± 6 beats/min, Table 3).

To assess insulin sensitivity, subjects underwent a euglycemic clamp. The results are shown in Table 4. Rates of basal endogenous glucose production were similar between the groups. However, the rate of insulin-stimulated glucose uptake was significantly higher in the lean subjects compared with the obese ($P < 0.05$). Although the obese subjects were slightly older, when glucose disposal was regressed against BMI, fat mass, and age using a stepwise model, BMI was the only variable that significantly influenced insulin-stimulated glucose uptake ($P < 0.05$), accounting for 50% of the variability among subjects. Age was not a significant factor.

Effect of a single bout of exercise. During the 48-min aerobic exercise bout (Fig. 1), heart rate was monitored continuously and recorded every 2 min. Exercise intensity was adjusted as necessary to maintain the target heart rate. Analysis of mean heart rate during each set showed that all participants reached the predicted heart rate, and no differences in heart rate during exercise were observed between groups (Table 3). Because heart rate and workload did not differ between the groups (Table 3), subjects exercised at the same relative and absolute intensities.

The expression levels of nuclear-encoded genes involved in mitochondrial biogenesis and function (primer sequences are given in Table 1) were analyzed using quantitative RT-PCR in muscle biopsy samples obtained at rest before the euglycemic clamp and 30 min and 5 h after exercise. At rest, there were no differences in mRNA expression between lean and obese groups for any gene other than AMPK-α_2, which was decreased by ~50% ($P < 0.05$; Table 5). To assess the effects of exercise, basal values were set to a value of 1.0. In the lean subjects, a single bout of exercise significantly upregulated

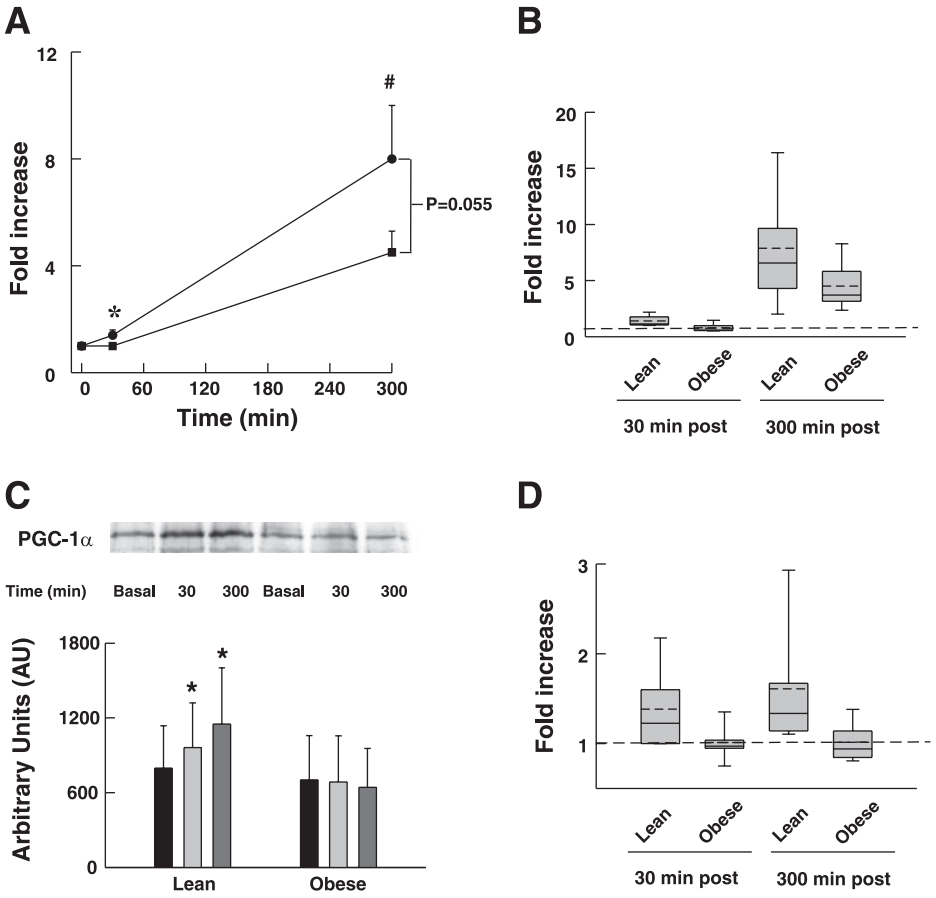

Fig. 2. Effect of exercise on peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) mRNA and protein expression. Expression of PGC-1α mRNA and protein levels were measured as described in METHODS. *A*: time course of PGC-1α mRNA response. Data were calculated using the $2^{-\Delta\Delta CT}$ method (see text) and were expressed as fold increase over baseline values for 7 lean (●) and 9 obese (■) subjects. Data are given as means ± SE. *B*: box-whisker plot of PGC-1α mRNA responses used in the 30- and 300-min postexercise periods for *A* (all basal values set to 1.0). The dashed line within the box shows the mean, the solid line within the box shows the median, the upper and lower limits of the boxes are the 95th and 5th percentiles, respectively, and the whiskers show the lower and upper data ranges. The horizontal dashed line at a value of 1.0 shows the preexercise value. *C*: PGC-1α protein abundance changes at 30 min (light gray bars) and 300 min (dark gray bars) after exercise, compared with basal values (solid bars) in lean and obese subjects. *D*: box-whisker plot of PGC-1α protein responses used in the 30- and 300-min postexercise periods for *C*. *$P < 0.05$, #$P < 0.01$ vs. basal values.

PGC-1α mRNA level as early as 30 min postexercise (Fig. 2*A*); this increase was then sustained for at least 5 h after exercise. Compared with lean subjects, obese insulin-resistant subjects had a delayed and reduced increase in PGC-1α mRNA level, even though relative exercise intensity, absolute work rate, and heart rate were the same. In particular, in obese subjects, no change compared with baseline was observed 30 min after exercise (Fig. 2*A*); however, 5 h after exercise, there was a significant increase in mRNA level compared with resting values. Compared with lean subjects, however, the increase in PGC-1α mRNA was significantly lower ($P < 0.05$) at 30 min in the obese group, and this trend was maintained at 5 h ($P = 0.055$). Using a stepwise regression analysis, we also evaluated whether confounding factors such as age, BMI, or fat mass were independently influencing our analysis. This analysis showed that age did not independently contribute to the variability in the response of PGC-1 mRNA to exercise. The variability of response to exercise of PGC-1α mRNA is shown using a box-whisker plot in Fig. 2*B*.

Having determined that exercise increased PGC-1 mRNA, next we assessed whether this increase was translated into higher protein abundance. Using immunoblot analysis, we found that the pattern of increase in PGC-1α protein mirrored that of mRNA, with the obese group having a delayed and blunted response compared with the lean control subjects (Fig. 2*C*). Variability in the response to exercise of PGC-1α protein abundance is shown in Fig. 2*D*.

In conjunction with a network of transcription factors, PGC-1α coordinates the expression of genes involved in aerobic energy metabolism. PGC-1α participates in regulating the expression of NRF-1 and NRF-2, which, in turn, control expression of regulators of mitochondrial DNA regulation, replication, and genes involved in the mitochondrial respiratory apparatus, such as cytochrome *c* oxidase (subunit VIc and VIIc) (24). Therefore, we investigated whether activation of PGC-1α was followed by further activation of these genes in response to exercise. Exercise by lean individuals significantly increased NRF-1 mRNA at 30 min and 5 h after exercise; no effect of exercise was observed in the obese subjects (Fig. 3*A*). In lean subjects, we also observed a subsequent activation of the downstream cytochrome *c* oxidase (subunit VIc) gene (Fig. 3*C*). NRF-2 and its downstream cytochrome *c* oxidase (subunit VIIc) were not activated in either group in response to a single bout of exercise at any time point analyzed (data not shown). These diminished responses in the obese subjects were consistent with the decreased response of PGC-1α to exercise. Variability in the responses to exercise of NRF-1 and cytochrome *c* oxidase, subunit VIc mRNA expression levels, are shown in Fig. 3, *B* and *D*.

Exercise also is a known regulator of AMPK. Recently, AMPK activation has been suggested to be involved in regulating expression of PGC-1α, thereby potentially playing a role in mitochondrial biogenesis or function (28). Because of the reduced response of PGC-1α to exercise in insulin-resistant subjects, we asked whether this could be due to decreased activation of AMPK. To answer this question, we examined the effect of exercise on the mRNA level of AMPK catalytic subunits α_1 and α_2 and of LKB1 complex (LKB1, MO25, and

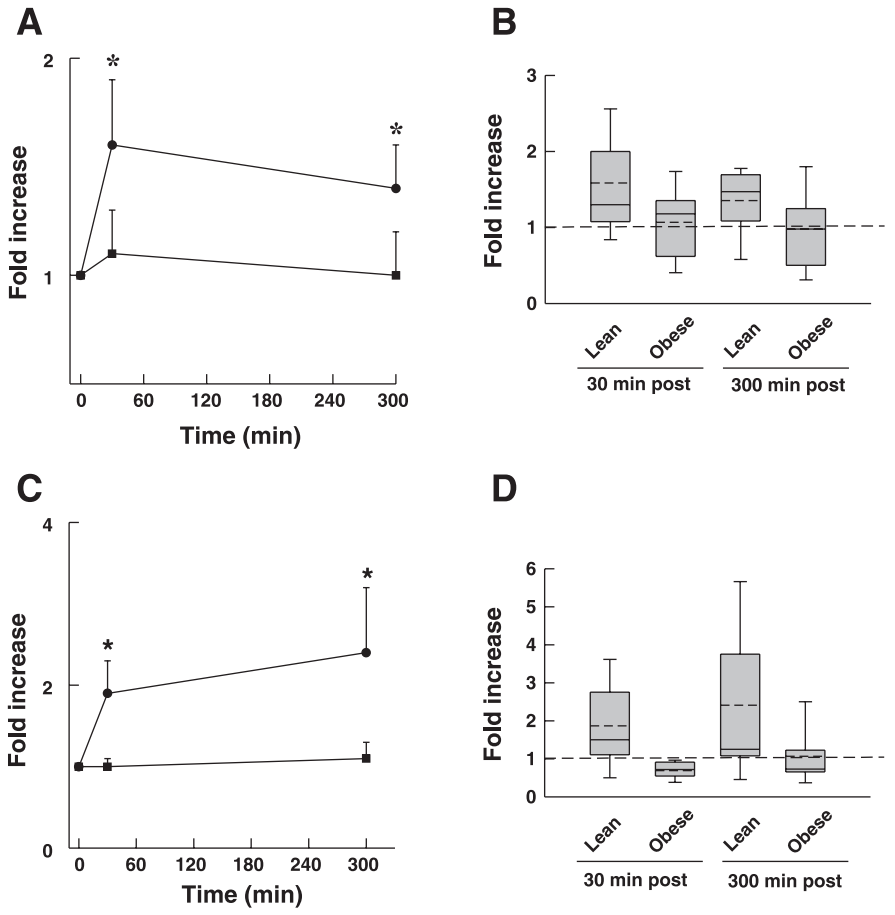

Fig. 3. Effect of exercise on nuclear respiratory factor-1 (NRF-1) and cytochrome *c* oxidase (subunit VIc) mRNA expression. *A*: time course of response of NRF-1 mRNA (mean ± SE) to exercise in 7 lean (●) and in 9 obese (■) subjects. β-Actin was used for normalization purposes. *B*: box-whisker plot of NRF-1 mRNA responses used in the 30- and 300-min postexercise periods for *A*. See Fig. 2 legend for description. *C*: time course of response of cytochrome *c* oxidase subunit VIc mRNA (mean ± SE) to exercise in 7 lean (●) and in 9 obese (■) subjects. β-Actin was used for normalization purposes. *D*: box-whisker plot of cytochrome *c* oxidase subunit VIc mRNA responses used in the 30- and 300-min postexercise periods for *C*. *$P < 0.05$ vs. basal values.

STRAD). LKB1 is one of the major kinases responsible for AMPK activation (10). There were no exercise-induced changes in the mRNA levels for any of these genes (Table 6), indicating that regulation at the level of gene expression was not involved. We, therefore, used immunoblot analysis to determine whether AMPK was activated by phosphorylation (Fig. 4*A*). Lean subjects showed a significant increase in Thr^{172} phosphorylation of AMPK-α within 30 min of the end of exercise, and this increase was maintained for at least 5 h. Obese individuals exercising at the same intensity had a transient activation of AMPK-α, reaching significance only at 30 min postexercise and decreasing thereafter (Fig. 4*A*). In keeping with the lack of increase in mRNA for AMPK-α, immunoblot analysis for the abundance of the catalytic subunit showed only a minimal increase (Fig. 4, *B* and *C*).

DISCUSSION

Several lines of evidence suggest that insulin resistance and impaired mitochondrial function are closely related. Studies using ^{31}P-NMR spectroscopy show decreased ATP turnover in insulin resistance (23), and enzymatic activity assays (25) as well as apparent structural changes (13) also reveal abnormalities. Studies using global gene expression analyses have shown mRNA expression of a cluster of genes encoding proteins involved in electron transport and oxidative phosphorylation is lower in insulin-resistant individuals, and that this concerted decrease possibly can be explained by decreased expression of PGC-1α (16, 21).

A number of studies have shown that an increase in PGC-1α expression mediated through AMPK signaling is important for the induction of mitochondrial biogenesis by exercise (3, 28, 29). A decrease in expression of PGC-1 and nuclear-encoded mitochondrial genes in insulin-resistant subjects could mean that these subjects have a lower level of physical activity or a reduced response of these genes to exercise. In the present study, we controlled for physical activity by studying only sedentary subjects who were matched for aerobic capacity ($\dot{V}O_{2peak}$) and peak work rate during a maximal exercise test. If there were an abnormality in mitochondrial biogenesis in insulin-resistant muscle, then it seems reasonable to hypothesize that exercise might not produce the same increase in PGC-1α expression in insulin-resistant individuals as it does in those who are insulin sensitive. To avoid any confounding effects of hyperglycemia, we also chose to test this hypothesis in normal glucose-tolerant, obese, insulin-resistant subjects, compared with healthy, lean controls. A single bout of exercise is known to increase PGC-1α gene expression (2, 29). To test the hypothesis that exercise differentially regulates gene expression in insulin-sensitive and insulin-resistant skeletal muscle, we used a single bout of exercise consisting of four sets of 12 min of cycling exercise, each of which included 2 min of high-intensity exercise (90% of $\dot{V}O_{2peak}$ heart rate).

Table 6. *LKB1, MO25, and STRAD mRNA expression at basal and postexercise*

	Lean			Obese		
Gene	Basal	30 min	5 h	Basal	30 min	5 h
LKB1	1.0	1.5±0.6	3.3±2.0	1.0	1.6±0.5	0.9±0.2
MO25	1.0	1.9±0.7	0.8±0.1	1.0	1.2±0.4	0.7±0.1
STRAD	1.0	1.3±0.3	1.1±0.3	1.0	1.4±0.5	1.6±0.5

Values are expressed as fold increase vs. baseline and given as means ± SE. Basal and postexercise mRNA levels were determined by RT-PCR in lean and obese subjects.

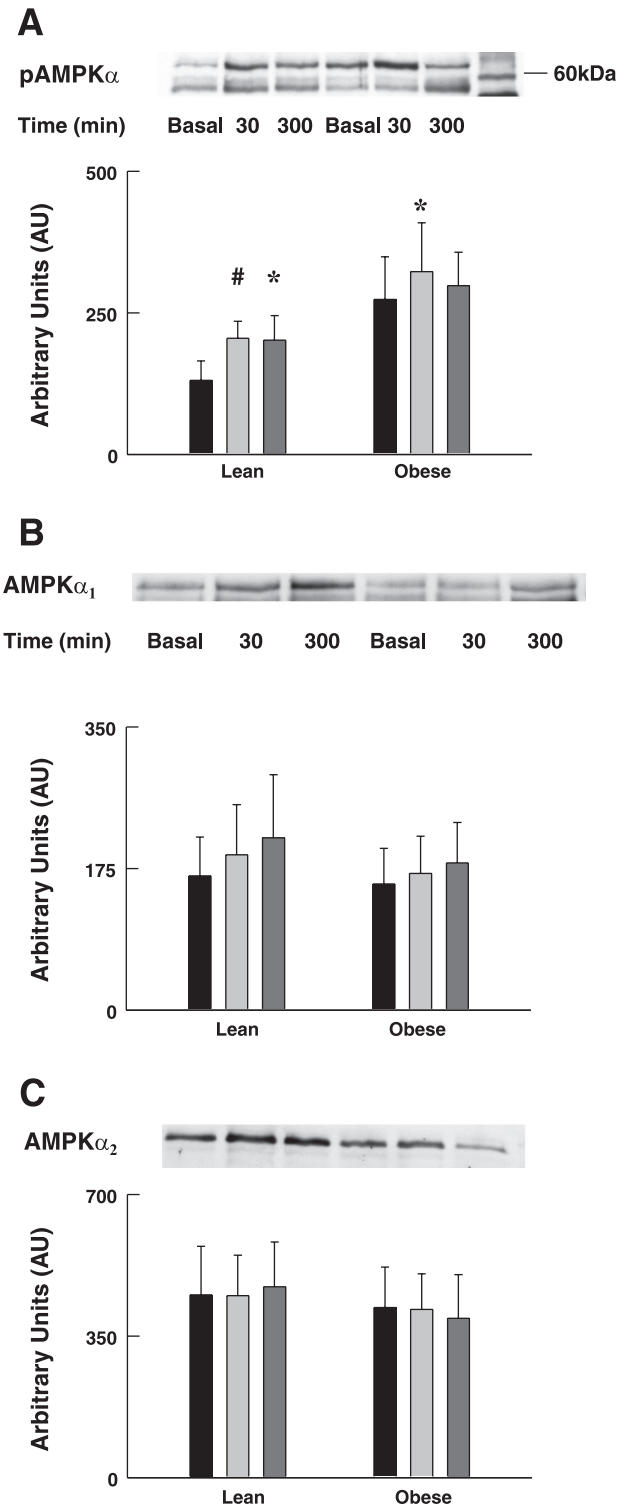

Fig. 4. Phospho-AMPK (pAMPK)-α and AMPK-α subunit protein content in lean and obese subjects. pAMPK (*A*), AMPK-α_1 (*B*), and AMPK-α_2 (*C*) subunits of protein abundance were measured in samples using immunoblot analysis, as described in METHODS. Values are means ± SE. *$P < 0.05$, #$P < 0.01$ vs. basal values.

At baseline, the majority of genes had similar expression levels in the lean and obese groups. The exception was AMPK-α_2 mRNA, which was reduced in the obese subjects. However, the small sample sizes of the groups in the present study temper conclusions. The results of this study show that, in insulin-sensitive subjects, exercise promptly increases PGC-1α mRNA. Within 30 min after the end of exercise, PGC-1α mRNA was significantly higher than basal levels, and this increase in mRNA for PGC-1α was magnified to about eightfold within 5 h after the end of exercise. In contrast, in the obese, insulin-resistant subjects, PGC-1α mRNA was not increased at 30 min following cessation of exercise. Although, after 5 h of rest, PGC-1α mRNA had increased in the insulin-resistant group, this increase was only one-half of that achieved by the insulin-sensitive subjects. These differences in gene expression changes were reflected in differences in changes in PGC-1α protein abundance, as determined using immunoblot analysis. In a recent study that used low- and moderate-intensity exercise (50 and 70% of $\dot{V}O_{2peak}$), it was reported that the response of PGC-1α mRNA to exercise was not significantly reduced in obese, insulin-resistant subjects (27). Using higher-intensity exercise (70–90% $\dot{V}O_{2peak}$), the results of the present study show that, in fact, exercise does not increase PGC-1α mRNA in a normal fashion in insulin-resistant muscle. The differences in the results of these studies are probably accounted for by the different exercise intensities that were used. Of note, the decrease in response of NRF-2 mRNA observed in that study (27) is consistent with a decreased PGC-1α mRNA response and also is supported by our findings. When the present findings are taken together with previous results (27), the evidence shows that a decreased ability of exercise to produce changes leading to mitochondrial biogenesis is associated with insulin resistance. For these and all responses observed in the present study, it must be noted that the sample sizes of the groups were small, as is typical for this type of study, and the results should be confirmed by additional experiments.

NRF-1 and NRF-2 are transcription factors that are under the regulatory control of PGC-1 (32) and are thought to mediate many of the effects of PGC-1. Expression levels of NRF-1 mRNA and one of its downstream activated genes, cytochrome *c* oxidase (subunit VIc), were increased in lean, insulin-sensitive controls, but not in obese, insulin-resistant individuals following exercise. These observations lend further weight to the notion that insulin resistance is accompanied by a reduced mitochondrial biogenic response to exercise and extend the previous findings (27) to the level of expression of nuclear-encoded mitochondrial genes, exemplified here by cytochrome *c* oxidase subunit VIc. It may be significant that both insulin resistance and the extent of response to exercise training are familial (1), and it is tempting to speculate that these phenomena are related.

Because AMPK activation by exercise has been implicated in the regulation of PGC-1α expression, we also examined the exercise-induced response of expression of AMPK-α_1 and -α_2 subunits as well as the LKB1 complex, which may be responsible for activation of AMPK (26). Exercise did not increase mRNA expression or protein abundance of any of these genes, verifying the results of a previous report (27). However, the exercise-induced increase in phosphorylation of AMPK was greater in insulin-sensitive subjects in the present study, consistent with the results for PGC-1α. Previous results (27) with lower intensity exercise showed that the response of AMPK activity to exercise was reduced in insulin-resistant muscle. The present results show that even high-intensity exercise may not be able to overcome this abnormality.

Taken together, these data suggest that, in insulin resistance, decreased AMPK phosphorylation and activation in response to exercise may lead to a diminished response of PGC-1α gene expression. The decrease in PGC-1α response, in turn, may lead to a decreased exercise-induced response of mitochondrial biogenesis to exercise in insulin-resistant subjects. A reduced ability to respond to exercise may then lead to reduced mitochondrial function, a decreased capacity of muscle for fat oxidation, accumulation of intramyocellular lipids, and subsequent inhibition of insulin signaling. In this manner, a vicious cycle reinforcing insulin resistance and diminished mitochondrial function may be established.

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