Exercise training increases electron and substrate shuttling proteins in muscle of overweight men and women with the metabolic syndrome

Dustin S. Hittel, William E. Kraus, Chuck J. Tanner, Joseph A. Houmard, and Eric P. Hoffman

1Research Center for Genetic Medicine, Children’s National Medical Center, Washington, District of Columbia; 2Division of Cardiology, Department of Medicine and Cell Biology, Duke University Medical Center, Durham; and 3Department of Exercise and Sport Science, and the Human Performance Laboratory and Diabetes/Obesity Center, East Carolina University, Greenville, North Carolina

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Hittel, Dustin S., William E. Kraus, Chuck J. Tanner, Joseph A. Houmard, and Eric P. Hoffman. Exercise training increases electron and substrate shuttling proteins in muscle of overweight men and women with the metabolic syndrome. J Appl Physiol 98: 168–179, 2005. First published September 3, 2004; doi:10.1152/japplphysiol.00331.2004.—Aerobic conditioned muscle shows increased oxidative metabolism or glucose relative to untrained muscle at a given absolute exercise intensity. The studies of a targeted risk reduction intervention through defined exercise (STRRIDE) study is an aerobic exercise intervention in men and women with features of metabolic syndrome (Kraus WE, Torga CE, Duscha BD, Norris J, Brown SA, Cobb FR, Bales CW, Annex BH, Samsa GP, Houmard JA, and Slentz CA, Med Sci Sports Exerc: 33: 1774–1784, 2001), with four muscle biopsies taken during training and detraining time points. Here, we expanded a previous study (Hittel DS, Kraus WE, and Hoffman EP, J Physiol 548: 401–410, 2003) and used mRNA profiling to investigate gene transcripts associated with energy and substrate metabolism in STRRIDE participants. We found coordinate regulation of key metabolic enzymes with aerobic training in metabolic syndrome (aspartate aminotransferase 1, lactate dehydrogenase B, and pyruvate dehydrogenase-α1). All were also quickly downregulated by detraining, although the induction was not an acute response to activity. Protein and enzymatic assays were used to validate mRNA induction with aerobic training and loss with detraining (96 h to 2 wk) in 10 male and 10 female STRRIDE subjects. We propose that training coordinately increases the levels of aspartate aminotransferase 1, lactate dehydrogenase B, and pyruvate dehydrogenase-α1 subunit, increasing glucose metabolism in muscle by liberating pyruvate for oxidative metabolism and, therefore, limiting lactate efflux. Serial measurement of fasting plasma lactate from 62 subjects from the same exercise group demonstrated a significant decrease of circulating lactate with training. We also found evidence for sex-specific molecular remodeling of muscle with ubiquinol-cytochrome c reductase core protein II, a component of mitochondrial respiratory complex III, which showed an increase after training that was specific to women. These biochemical adaptations complement existing molecular models for improved glucose tolerance with exercise intervention in prediabetic individuals.

microarray; aspartate aminotransferase; lactate dehydrogenase

TRAINED AND UNTRAINED SKELETAL muscle possesses an inherent and dynamic capacity for metabolic flux. This is most evident in the 10-fold increase in metabolic rate with moderate to intense physical activity in untrained but otherwise healthy individuals. Trained athletes show even greater proportional increases in the postabsorptive state, skeletal muscle is fueled primarily by the oxidation of fatty acids, which, although relatively slow, produces a high ATP yield. As the intensity of exercise increases, a gain in muscle glycolytic flux, mainly from glycogen, provides most of the substrate for mitochondrial respiration, whereas β-oxidation of fatty acids occurs at rates similar to those at rest or lower (8, 49). Glycogenolysis and glycolysis can generate ATP more rapidly than oxidative metabolism but do so at the expense of metabolic economy, generating only 2 or 3 ATP molecules per plasma glucose or glycogen-derived glucosyl unit, respectively, compared with the 36–38 produced by oxidative metabolism (5).

Skeletal muscle from untrained subjects is proportionately more reliant on glycogenolysis and glycolysis to generate ATP because it is less perfused and the mitochondrial reticulum is less elaborated (5, 24). This also limits the ability of muscle to utilize both blood-borne free fatty acids and intramuscular triglycerides as substrates for energy metabolism (5). However, when adapted to habitual aerobic exercise, muscle is able to utilize the oxidative catabolism of both substrates to generate ATP at higher absolute workloads than in the untrained state (5, 16). This is particularly relevant to the prediabetic metabolic syndrome, which is characterized by impaired glucose tolerance, insulin sensitivity, and mild to severe lipid abnormalities (26), and highlights the importance of exercise in limiting or reversing its progression to Type 2 diabetes.

Traditionally, studies of the metabolic remodeling of muscle have relied on biochemical and physiological measurements. Although these have been highly informative, it is difficult to parallelize data generation and thereby begin to develop a broader picture of muscle remodeling after training. Newly emerging mRNA profiling and proteomic methods can impart a “genomewide” assessment of muscle plasticity. The reproducibility and redundancy of the Affymetrix GeneChip platform has been particularly effective in producing high-quality gene expression data from skeletal muscle and in generating new hypotheses about its regenerative capacity and phenotypic plasticity (12, 13, 20, 21, 53). In a previous microarray-based pilot study using three male subjects from the studies of a targeted risk reduction intervention through defined exercise (STRRIDE) exercise intervention study, our laboratory (20) found that trained muscle produces large amounts of clot-destroying fibrinolytic proteins. This study showed that the systemic fibrinolytic state was favorably modulated by chronic exercise. This finding was particularly significant because the
metabolic syndrome is an independent risk factor for cardio-
vascular disease (22, 51). To explore this model further, we
applied similar methods toward a systematic evaluation of
skeletal muscle transcriptional remodeling in response to
chronic exercise training of overweight individuals with char-
acteristics of the metabolic syndrome. Toward this end, we
studied biopsies from three female and three male STRRIDE
subjects each in the “high” exercise group (2,200 kcal/wk, 3
mo ramp up, 6 mo training) (26). We analyzed four muscle
biopsies from each subject: one at entry, and three after 9 mo
of aerobic training [24 h after the last bout, 96 h after the last
bout, and 14 days after the last bout (detraining)]. We then used
a validation set of seven men and seven women from the same
group, as well as plasma samples (for fasting lactate) from
larger numbers of subjects. Our rationale for studying this
population was to increase understanding of aspects of the
metabolic syndrome in participants in STRRIDE where serum
lipid profiles and insulin resistance were normalized after the
exercise intervention (22, 25).

METHODS

STRRIDE study design and considerations. All subjects provided
written, informed consent. This study was performed in accordance
with the Declaration of Helsinki and approved by the Institutional
Review Board of the Duke University Medical Center (26). We have
previously reported expression profiling of three men using U95A
microarrays (12,000 probe sets). Here, we extended this analysis to
the newer generation U133A microarrays (18,400 probe sets) and
to three women from the same exercise group (6 subjects; 4 biopsies
each; 24 U133A microarrays). We also studied a validation set of
muscle biopsies, using seven women and seven men participating in
the same high-dose and high-intensity aerobic exercise group [2,200
kcal/wk at 65–80% peak VO\textsubscript{2}] of the STRRIDE study;
three of each sex were used for mRNA profiling and seven for protein
validation of mRNA results.

Women and men ranged from 40 to 64 yr of age, were sedentary
(exercise < once weekly; peak VO\textsubscript{2} 25.8–34.6 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}) and
overweight (body mass index 27.9–36.1), and exhibited signs of
mild to moderate lipid abnormalities (low-density lipoprotein 132–
163 mg/dl and high-density lipoprotein 27–40 mg/dl). Skeletal mus-
cle biopsies were obtained from the percutaneous needle biopsy tech-
nique using 1% lidocaine without epinephrine (26). Four serial vastus
lateralis muscle biopsies (100–200 mg) were obtained with a triple
pass of the bioprime needle: one on entry into the study, one after 9
mo (3-mo ramp up, 6-mo training) of exercise training (within 24 h
after the last bout of exercise), and two detraining time points 96 h (4
days) and 14 days (2 wk) after the last exercise bout. Biopsy samples
were flash frozen for histological analysis, and an average of 25
mg/biopsy were shipped on dry ice for gene expression profiling and
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Produced by endogenous parallel experiment to determine background levels of activity protein...

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170 METABOLIC REMODELING OF TRAINED MUSCLE

Lactate dehydrogenase isozyme analysis. A nondenaturing gel electrophoresis method was adapted for skeletal muscle lactate dehydrogenase (LDH) isozyme analysis (40, 47). Briefly, an 8% resolving gel and a 3% stacking gel were polymerized in the absence of SDS. Twenty micrograms of protein suspended in native loading buffer (3 ml glycerol, 0.6 ml 50× running buffer, 6.4 ml H2O, bromophenol blue) were run in a cold room at 30 mA (stack) and then 40 mA (resolving) until the gel tracking dye was ~1 cm from the bottom. Gels were washed with running buffer (50× diluted, 7.5 g Tris base, 36 g glycine, water to 250 ml) and then incubated in the dark at 37°C in an 80 mM potassium phosphate buffer (pH 7.4) with 0.1 M lithium lactate, 1 mM nitroblue tetrazolium, and 25 μM phenazine methosulfate. Gels were then scanned and dried for storage. Expression levels were quantified from scanned X-ray film images by use of UNSCANIT automated digitizing system (Silk Scientific, Orem, UT).

Aspartate aminotransferase assay. The kinetic assay for aspartate aminotransferase (GOT) couples the products of endogenous GOT with exogenous malate dehydrogenase (MDH) and the concomitant oxidation of NADH (2). GOT catalyzes the transfer of an amino group between l-aspartate and 2-oxoglutarate, forming oxaloacetate (OAA), which is subsequently reduced with NADH (in a 1:1 ratio) in the presence of MDH, forming oxidized NAD+, which can be monitored at 340 nm. GOT assay reagent (all reagents from Sigma) was composed of 12 mM 2-oxoglutarate, 200 mM l-aspartic acid, 0.19 mM NADH, and 600 U/l MDH in a 100 mM sodium phosphate buffer, pH 7.8. Reagent was prewarmed to 37°C for 1 min, and then the absorbance was blanked against distilled water. In a 96-well Labsystems Multiskan MCC/340 microplate reader (Fisher), 200 μl of reagent and 10 μg of protein were combined, and the initial absorbance at 340 nm was taken before and after 1-min incubation (linear portion of curve) for a total of 2 min. l-Aspartate was omitted in a parallel experiment to determine background levels of activity produced by endogenous α-keto acids. For the purpose of this experiment, exogenous GOT was purchased (Sigma) to determine the linear range of the assay by a microplate procedure.

Plasma analysis. A fasting venous blood sample was obtained, and plasma was frozen at −80°C for the subsequent determination of lactate. Plasma was analyzed for lactate with an oxidation reaction (YSI 2300 STAT Plus glucose and lactate analyzer, YSI, Yellow Springs, OH) (6, 22).

Statistics and data analysis. Unless otherwise stated, all statistical analysis were conducted by using a paired Student’s t-test. Significance for all comparisons was set at P < 0.05. Data are represented as means ± SE.

RESULTS

mRNA expression profiling. Analysis of mRNA expression patterns in the four muscle biopsies from each individual over the exercise training-detraining time course identified genes whose expression levels were exercise responsive and shared by the three women, three men, or all six subjects. Probe set algorithms employed were both MAS 5.0 and dCHIP difference model, with validation using a 14-subject (4 biopsies/subject) validation set (7 men and 7 women from the same exercise group).

Of the 18,400 genes probed using the Affymetrix human U133A arrays after scaling, correcting for background, and filtering using flags (present calls) and statistical analysis (P < 0.05 for paired biological replicates), 429 genes were differentially expressed in skeletal muscle of women after 9 mo of aerobic exercise training on the basis of P value with no adjustment for multiple testing. We first focused on the gene expression changes that showed an average 1.5-fold change or greater (136 of 492; 28%). Using the same filtering criteria for the male skeletal muscle samples, we found significantly fewer expression changes (182 genes with paired t-test <0.05 in men compared with 492 in women). When we filtered for fold change, 73 genes showed upregulation and 23 were downregulated with a relative fold change ≥1.5 (about one-half the changes found in women). These data suggest that women exhibit a more pronounced transcriptional response to resistance training than men. A full list of statistically significant aerobic exercise-responsive genes in the men and three women (STRRIDE U133A Genes.xls) is available for download as supplementary material at: http://microarray.cnmcresearch.org/pgradationtable.asp under the project name Wkraus STRRIDE Study, or via our PEPR database: http://pepr.cnmcresearch.org/browse.do?action=list_prj_exp&projectId=192. The latter includes gene-based queries of either longitudinal or cross-sectional data via the “chart” function. In addition to our “in house” database, a full data set was submitted to Gene Expression Omnibus under the accession number series GSE12955.

Metabolic remodeling. We focused on mRNAs that encoded proteins involved in substrate and energy metabolism with the goal of providing insight into the pathophysiology of the metabolic syndrome and the benefits of exercise intervention. Genes discovered using dCHIP or MAS5.0 included UQCRCC2, glycogen synthase 1, ALAS1 (heme synthesis), GOT2 [malate-aspartate (MA) shuttle], IGFBP7, the mitochondrial isoforms of creatine kinase and aconitase and others (supplementary data) (23, 34). GOT1 and LDHB were detected by using both dCHIP and GeneSpring. Some genes, such as UQCRCC2, ALAS1, and IGFBP7, were differentially expressed in a single sex, whereas GOT1, LDHB, PDHA1, aconitase, creatine kinase, and glycogen synthase 1 were upregulated in both sexes. Several of these genes, particularly those exhibiting gender and/or fiber-type specificity, are currently being investigated.

We focused on a subset of metabolic proteins of known function that were strongly induced by training in both men and women, or showed sexual dimorphism in this response [paired t-test P < 0.05 between time point 1 (entry) and time point 2 (9 mo training)]. Each was validated in a set of muscle biopsies from the same exercise group by immunoblot and/or enzyme activity. The validation set included the three individ-
uals of each sex used for microarray analysis (24 biopsies) and an additional set of 7 male and 7 female subjects (56 biopsies).

*Aspartate aminotransferase 1 expression and total activity.*

Cytosolic aspartate aminotransferase, also known as glutamate-oxoglutarate transaminase (GOT1), is the rate-limiting enzyme in the MA shuttle (2). The three known NADH shuttles function in part to regenerate the NAD⁺, which in turn is needed to drive the glycolytic production of pyruvate destined for the TCA cycle. It is worth noting that the inhibition of GOT activity significantly decreases the oxidative catabolism of glucose and increases lactate production (7). In addition, the activities of MA shuttle enzymes have previously been shown to increase with exercise training in young healthy men, but never in the context of the metabolic syndrome, and women have not been studied (42).

GOT1 mRNA showed strong induction with training in women and to a lesser extent in men; this was most evident when normalizing to mean expression levels across all time points in the three women (Fig. 1A) and three men (Fig. 1B) studied by expression profiling. During the 96-h and 14-day detraining time points, steady-state GOT1 mRNA levels dropped nearly to baseline, pretraining levels in both men and women. These data suggest that the expression of this gene in untrained muscle is sexually dimorphic, with men showing considerably higher basal levels pretraining than women.

To validate the response of GOT1 expression to aerobic training, we studied protein levels in biopsies of both the three women (Fig. 1A) and men (Fig. 1B) used for microarray analyses and in the seven female and seven male validation biopsy set. Considering all 10 subjects, the amount of immunologically reactive GOT1 protein in women increased significantly (4.7 ± 0.5-fold) compared with the 2.3-fold increase detected by Affymetrix analysis (Fig. 1A). This is consistent with our previous findings that, although protein levels do not directly correlate with mRNA level, the direction of the change (up or down) was consistent with Affymetrix data (20). In the 10 men studied (Fig. 1B), the amount of immunologically reactive GOT1 protein increased but less dramatically and with more variance than was seen in the women (2.7 ± 1.2-fold). Although smaller in magnitude, the change in GOT1 protein in men was statistically significant and in agreement with the lower 1.6-fold change detected by Affymetrix analysis (Fig. 1B). In the 96-h and 2-wk detraining time points, GOT1 protein levels decreased ~50% in both men and women relative to the 9-mo training point (Fig. 1, A and B). The drop in protein levels was not as dramatic as the decrease in mRNA levels; however, this is commonly observed, as most proteins have longer half-lives than mRNAs.

Steady-state protein levels detected by immunoblotting do not necessarily reflect enzymatic activity. We, therefore, studied GOT1 enzymatic activity in muscle biopsy lysates from the validation muscle biopsy set (7 men, 7 women). We studied total GOT activity (cytosolic plus mitochondrial), rather than cytosolic alone, because pure cytosolic preparation was not possible given the biopsy size and flash-frozen preservation technique used. The mRNAs of GOT2 and other proteins involved in the MA shuttle were also increased with training but less significantly than GOT1 (supplementary data). Total skeletal muscle GOT activity increased significantly in women (0.107—0.223 U/mg protein, \( P = 0.045 \)) and to a lesser magnitude in men (0.26—0.390 U/mg protein, \( P = 0.056 \)) after exercise training (Fig. 1C). Enzymatic activity data mirrored GOT1 protein abundance in (male and female) muscle before and after training.

*Lactate dehydrogenase B expression and LDH isozyme analysis.*

The lactate dehydrogenase complex (LDH) catalyzes the near-equilibrium interconversion of pyruvate to lactate with concomitant conversion of NADH to NAD⁺. Lactate dehydrogenase B (LDHB) or heart-type isof orm exhibits a higher affinity for lactate than lactate dehydrogenase A (LDHA) or muscle-type isof orm and is preferentially expressed in highly oxidative tissues (6). In the context of an enhanced capacity for NAD⁺ regeneration (via the MA shuttle), increased LDHB expression would increase the economy of glucose metabolism in trained muscle, making it better able to respond to insulin-stimulated glucose uptake. Although changes in LDH isozyme distribution have been reported previously in highly trained athletes, it has not been investigated in the context of exercise intervention and the metabolic syndrome and the coordinated upregulation of pathway members LDHB and GOT1 (5).

In the 10 women studied (Fig. 2A), the amount of immunologically reactive LDHB protein increased significantly (6.7 ± 2.1-fold) after exercise training and then decreased incrementally after 4 days and 14 days of posttraining to near untrained levels. The LDHB protein level changes in women were greater in magnitude yet closely mirrored the trend of Affymetrix LDHB transcript levels (Fig. 2A). The amount of immunologically reactive LDHB protein also increased significantly (8.1 ± 0.5-fold) after exercise training in the 10 men studied. These levels persisted or dropped slightly after 4 days of detraining and then returned to near untrained levels 14 days posttraining (Fig. 2B).

LDHA and LDHB coexist in most cells in different amounts on the basis of the metabolic demands placed on the tissue or organ (32). Given also the limited amount of biopsy material, we decided that a qualitative analysis of LDH isozyme distribution would provide more information about cell autonomous changes in the oxidative state of trained myofibers (Fig. 2C). All subjects [represented by *male subject 1* (M1) in this figure] showed a relative decrease in the activities of the LDH5 isozyme and parallel increases in LDH3 and LDH4 in response to aerobic exercise training (Fig. 2C). This is likely the consequence of increased LDHB protein abundance (Fig. 2, A and B) because LDH3 and LDH4 isozymes contain one and two LDHB subunits, respectively. This trend was observed in skeletal muscle from both men and women and is thought to indicate an overall trend toward enhanced expression of genes of oxidative metabolism in exercise-trained skeletal muscle (32).

*Serum lactate analysis.*

The increased levels of GOT1, LDHB, and PDHA1 suggested an increased ability of the muscle to metabolize glucose more efficiently, producing less lactate as a result. This suggested that serum lactate might decrease as a function of aerobic training, in that muscle could better utilize endogenous lactate and thus release less into the serum. Fasting serum lactate levels, before and after exercise training, were quantified in 36 men and 26 women from the same high-dose, high-intensity STRRIDE exercise group. By use of a paired statistical analysis, changes in fasting lactate were decreased in both men and women but reached statistical significance only in women (Fig. 3A). When data are combined irrespective of gender, changes in fasting lactate were signifi-
cant and rapidly reversible with cessation of regular exercise in the detraining time points (Fig. 3B).

Pyruvate dehydrogenase \( \alpha \)-subunit expression. Increased glucose and lactate metabolism via LDHB and GOT1 should lead to greater flux of intracellular pyruvate, and thus the potential for increased activity of pyruvate dehydrogenase (PDH) to shuttle carbon chains into the mitochondria to drive the oxidative phosphorylation of ATP. In fact and very re-
Recently, the pyruvate dehydrogenase α1-subunit (PDHA1) has been shown to increase in muscle with training (28); however, this previous study was in young healthy volunteers, and the extent of transcriptional changes previously observed was considerably less than what we observed here in individuals with the metabolic syndrome.

In the 10 women studied, the amount of immunologically reactive PDHA1 protein increased significantly (2.1 ± 0.9-fold) after exercise training and then decreased rapidly to pretraining levels after 14 days of detraining (Fig. 4A). As well, in the 10 men, the amount of immunologically reactive PDHA1 protein increased significantly (4.1 ± 1.5-fold) after exercise training, persisted or dropped slightly after 4 days of detraining, and then also decreased rapidly after 14 days of detraining (Fig. 4B). Western blot data indicated that untrained women have relatively high concentrations of PDHA1 compared with similarly untrained men, opposite the trend observed for GOT1. As well, the protein levels of PDHA1 closely followed the Affymetrix transcriptional profile of the corresponding gene in both men and women over the exercise training and detraining cycle.

**Fig. 2.** mRNA, immunoblot, and isozyme analysis of lactate dehydrogenase B (LDHB) protein levels in female (A) and male (B) STRRIDE skeletal muscle over a 9-mo aerobic training and 96-h and 2-wk detraining time series. Values are means ± SE. *Significant difference (paired Student’s t-test, *P* < 0.05, *n* = 10) relative to control time point. C: lactate dehydrogenase (LDH) isozyme analysis of STRRIDE skeletal muscle over a 9-mo aerobic training and 96-h and 2-wk detraining time series. Shown is a LDH isozyme in-gel activity stain of a female subject that was most representative of the skeletal muscle LDH isozyme pattern observed in men and women with exercise training. Although amounts of protein were loaded into each lane, there is a distinct shift to the more aerobic LDH4 (M3H1) and LDH3 (M2H2) isozymes with a concomitant drop in the abundance of the LDH5 (M4). The decrease in LDH5 and increase in LDH4 were consistent throughout the training and detraining time series.
are short lived and return to pretraining values without the training stimulus. The control time point. Trend indicates changes in fasting lactate with training and 96-h and 2-wk detraining time series. *Significant difference (repeated-measures ANOVA, 

plasma lactate of male and female STRRIDE subjects over the 9-mo aerobic training and 

women. *Significant difference (P<0.05, n=59) relative to control time point. Trend indicates changes in fasting lactate with training are short lived and return to pretraining values without the training stimulus.

UQCRC2. Ubiquinol cytochrome c reductase core protein 2 (UQCRC2), one of the core nuclear-encoded components of the mitochondrial electron transport complex III, exhibited significant upregulation in women but not men with training. UQCRC2 was also chosen because it is likely representative of increased mitochondrial density and, hence, oxidative capacity associated with aerobic exercise training in muscle. As with PDH and GOT, many mitochondrial enzymes were similarly upregulated in trained muscle, although most did not exhibit gender-specific expression (supplementary data). To our knowledge, no previous report has studied UQCRC2 during training or investigated sexual dimorphism in mitochondrial electron transport chain components.

The protein level of UQCRC2 in the 10 women studied showed a strong increase in immunologically reactive UQCRC2 protein with training (7.1 ± 1.1-fold increase). However, unlike GOT1, LDHB, and PDHA1 proteins, the relative expression of UQCRC2 remained high even after 14 days of detraining (Fig. 5A). In men, there were no significant changes in UQCRC2 protein levels after training, indicating a gender difference in exercise responsiveness and in baseline protein levels in trained and detrained skeletal muscle (Fig. 5B).

In silico analysis of glucocorticoid-induced gene expression. Our facility maintains a public resource of expression profile data containing 1,024 human, mouse, and rat Affymetrix GeneChip expression profiles, which are subject to the same quality and procedural controls [Public Expression Profiling Resource (PEPR)]. Our Oracle-based PEPR data warehouse includes a time series query analysis tool, SGQT, that allows for the dynamic generation of graphs and spreadsheets showing the action of any transcript of interest over time (11). This resource can be accessed at http://pepr.cnmcresearch.org. GOT1, LDHB, and PDHA1 have been shown to contain glucocorticoid response elements (GREs) in the promoters of their respective genes (31, 43, 44, 46). We, therefore, queried two extended microarray time series (rat liver and skeletal muscle) for their in vivo responses to a single bolus dose of methylprednisolone (1). There was no probe set corresponding to the rat homolog for human PDHA1 on this version of the Affymetrix rat chip. Surprisingly, neither LDHB nor GOT1 responded significantly to the methylprednisolone bolus in skeletal muscle. In liver, however, there was a 3.5-fold increase in GOT1 expression 6–7 h after the methylprednisolone bolus levels, which rapidly returned to normal after 15 h (Fig. 6). There was also a two- to fourfold change in LDHB expression in liver 2–7 h after methylprednisolone bolus that also returned to normal levels after 15 h as expected (Fig. 6). The surprising lack of glucocorticoid-induced expression of GOT1 and LDHB in rat skeletal muscle may reflect tissue-specific or species-specific response to endogenous (cortisol) vs. exogenous (methylprednisolone) glucocorticoids.

Insulin levels. Fasting plasma insulin was measured in female (n = 26) and male (n = 36) STRRIDE participants before and after 9 mo of exercise training (Fig. 7). In both genders, levels of fasting insulin dropped with exercise training, possibly indicating an increase in insulin sensitivity.

DISCUSSION

The data presented here used longitudinal muscle biopsies from volunteers with symptoms of metabolic syndrome from the STRRIDE study (26) to show that key proteins involved in both glycolytic and oxidative metabolism are coordinately induced with aerobic training, that most show rapid loss of expression with detraining (by 14 days), and that there are sex differences in basal levels of some. Although much of this remodeling has been shown in specific instances previously, the alterations we observed in the metabolic syndrome subjects were generally considerably more dramatic than previously observed, and the use of mRNA expression profiling in a longitudinal cohort of men and women allowed us to observe...
the coordinate regulation and deregulation of these biochemical pathways (45). These observations have clinical significance because they demonstrate a preservation of adaptation to physical training in metabolic syndrome patients and show that the beneficial training effect, although lasting for days, is eventually lost by 2 wk. This emphasizes the importance of exercise prescriptions for metabolic syndrome patients and that exercise needs to be maintained on a weekly basis throughout the year.

Also, when compared against previous microarray studies on the acute effects of exercise on rat muscle (12), no significant changes were noted in the metabolic genes described herein. This suggests that these changes in gene expression are the result of exercise training rather than an acute bout effect. Integrating previous literature with our data elaborates on existing models of glucose and lactate metabolism by proposing biochemical cooperation and pretranslational coordination between the MA shuttle, LDH, and PDH complexes and links this with training-associated changes in fasting serum lactate.

Our model of the metabolic remodeling of skeletal muscle in metabolic syndrome patients (Fig. 8) describes an adaptive increase in the integration of glucose oxidation in response to the rapid changes in cellular energy demand with habitual

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Fig. 4. Immunoblot analysis of pyruvate dehydrogenase α1-subunit (PDHA1) protein levels in female (A) and male (B) STRRIDE skeletal muscle over 9-mo aerobic training and 96-h and 2-wk detraining time series. Values are means ± SE. *Significant difference (paired Student’s t-test, $P < 0.05$, $n = 10$) relative to control time point.

Fig. 5. Immunoblot and analysis of ubiquinol cytochrome c reductase core protein 2 (UQCRC2) protein levels in female (A) and male (B) STRRIDE skeletal muscle over a 9-mo aerobic training and 96-h and 2-wk detraining time series. Values are means ± SE. *Significant difference (paired Student’s $t$-test, $P < 0.05$, $n = 10$) relative to control time point. Changes in the abundance of this mitochondrial electron transport protein were sex specific. It is uncertain whether this reflects sex-specific changes in muscle mitochondrial abundance with training.
aerobic exercise. Specifically, these adaptations are mediated by increased protein and activity levels of GOT1, LDHB, and PDHA1 and, in women only, UQCRC2.

Cytosolic aspartate aminotransferase, also known as glutamate-oxoglutarate transaminase 1 (GOT1), is the rate-limiting enzyme in the MA shuttle while also providing carbon skeleton intermediates for gluconeogenesis in the liver and kidney (2). In vivo, GOT transfers an amine from aspartate to 2-oxoglutarate (α-ketoglutarate), producing OAA and glutamate. At this point, the OAA may be used as a gluconeogenic precursor (via PEPCK) or as an electron shuttle across the inner mitochondrial membrane when reduced by MDH (2, 31). Collectively, the three known cellular NADH shuttles provide a conduit for the transfer of reducing equivalents across the inner mitochondrial membrane. The other two NADH shuttles include the glycerol phosphate shuttle, which is less efficient than the MA shuttle (17), and the recently proposed intracellular lactate shuttle (9). Regardless of the specific shuttle, the regeneration of cytosolic NAD$^+$ is critical for sustaining a high rate of glycolytic ATP production. However, when glycolytic NADH generation exceeds the electron shuttle capacity (i.e., during intense exercise), pyruvate is increasingly favored as an electron acceptor and is rapidly reduced to lactate and temporarily (if it is reoxidized in the muscle) or permanently (if it is exported into circulation) sequestered from the high-ATP-yielding oxidative metabolism within the mitochondria (9, 19).

We propose that, in untrained metabolic syndrome patients, low levels of GOT1 protein and activity in skeletal muscle limit its capacity for glycolytic flux in the absence of significant lactate production. This is particularly relevant during higher intensity exercise, such as that used in this study, when muscle favors glucose as a substrate, and the lack of sufficient NADH shuttle capacity would drive the production of lactate. For this reason, we believe that untrained skeletal muscle becomes a net producer of lactate, which likely contributes to elevated fasting lactate levels in untrained individuals and impaired glucose tolerance (Fig. 3). Conversely, increased GOT1 protein and activity in trained muscle should explain, in part, training-associated decreases in fasting lactate levels in

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**Fig. 6.** In silico analysis using a single gene query of GOT1 and LDHB expression in rat liver for their responses to a single bolus dose of methylprednisolone. Shown on the ordinate is the Affymetrix-derived average difference level and on the abscissa the time (in hours) after the methylprednisolone bolus.

**Fig. 7.** Fasting plasma insulin of female ($n = 26$) and male ($n = 36$) STRRIDE participants before and after 9 mo of exercise training. Values are means ± SE. In both groups, there was a significant change in fasting plasma insulin indicative of increased insulin sensitivity after training. *Significant difference ($P < 0.05$ paired Student’s t-test) in both women and men.
women and men, although ideally this should be determined by a lactate threshold or stress test. This may be significant because consistently elevated levels of circulating lactate stimulate hepatic glucose production, which, when combined with peripheral insulin resistance and decreased metabolic clearance of glucose (both characteristics of the metabolic syndrome), leads to increasingly severe bouts of hyperglycemia typical of Type 2 diabetes (4, 27, 29).

Increased levels of GOT1 activity have been associated with increased maximal \( \dot{V}_{O_2} \) of exercise-trained humans and animals (33, 38); however, the data presented here show that basal levels of GOT1 are very low in subjects with features of metabolic syndrome. At the exercise intensities associated with the ascertainment of peak or submaximal \( \dot{V}_{O_2} \) measurements, muscle is highly dependent on glycolysis and glycogenolysis (8). The production of lactate rapidly limits substrate entry into the (oxidative) TCA cycle, particularly when muscle glycogen supply is limiting because of lack of training and insulin resistance. Our model then describes how the enhanced capacity of the MA shuttle (via GOT1) liberates pyruvate for oxidative ATP production in the TCA cycle. This may account also for the up to 39% increase in the enhanced capacity for glycolytic flux in the absence of net lactate production. Because inhibition of GOT activity increases lactate production, we propose that the coordinate induction of LDHB and GOT1 increases the economy of muscle glucose oxidation by driving instead the production of pyruvate, which is channeled to the oxidative production of ATP via the TCA cycle and mitochondrial electron transport chain.

The PDH complex has a molecular weight of \( \sim 7 \) million and is composed of multiple copies of three enzymes: pyruvate decarboxylase (E1), hydrolipoyl transacetylase, and dihydrolipooyl dehydrogenase (46). The E1 enzyme is itself a complex, a heterotetramer of two \( \alpha \)- and two \( \beta \)-subunits. The E1-\( \alpha \) subunit (PDHA1) plays a key role in the function of the PDH complex because it contains the E1 active site and is thought to be the rate-limiting step in the conversion of pyruvate to acetyl-CoA (30). Recently, PDHA1 expression in muscle has been shown to increase in response to 8 wk of aerobic exercise training in healthy young men (28). This responsiveness of PDHA1 to exercise training, coupled with our observations of its increase with training and decrease with detraining (Fig. 4), suggests that its expression levels and perhaps the activity of the PDH complex itself are impaired in obese and/or glucose-intolerant individuals.

Taken together, the increased abundance and, in some cases, the increased activity of PDHA1, LDHB, and GOT1 likely reflect the enhanced integration and economy of glycolysis and the TCA cycle within exercise-trained skeletal muscle of obese individuals with characteristics of the metabolic syndrome.

The data presented here also provide new insights into gender disparity with regard to baseline muscle metabolism and subsequent remodeling with training. Specifically, UQCRC2 was discovered to be upregulated in women but not men according to dCHIP analysis. UQCRC2 is one of the core proteins of the mitochondrial electron transport chain complex III, which transfers electrons from ubiquinone to cytochrome \( c \) by using the change in electron energy state to translocate...
protons across the inner mitochondrial membrane (17, 35). The striking differences in exercise-responsive protein levels of UQRC2 between women and men may simply reflect higher basal UQRC2 protein content in men or possibly significant gender differences in mitochondrial adaptation to exercise (16). In addition to UQRC2, many genes associated with the mitochondrial electron transport chain, mitochondrial structure and function, and metabolism were upregulated in skeletal muscle from exercise-trained muscle of women and men (supplementary data), indicating a general increase in mitochondrial density and volume with exercise training (24). Realizing this, we integrated UQRC2 into our metabolic model to reflect this overall increase in mitochondrial density and how this would effectively increase the capacity for oxidative metabolic flux by providing more surface area for the translocated NADH and pyruvate to power the oxidative phosphorylation of ATP. A recent microarray survey of skeletal muscle from insulin-resistant and diabetic individuals described the coordinated downregulation of mitochondrial genes of oxidative and energy metabolism (35, 52). Not only are these findings in agreement with our model, but they may also explain, in part, some of the beneficial effects of habitual exercise in ameliorating these conditions in metabolic syndrome patients.

A true coordinated cluster of genes must be regulated by one or several common transcriptional response or promoter elements. LDHB, GOT1, and PDHA1 have all been reported to be glucocorticoid responsive, and all show GREs in their respective gene promoters (31, 43, 46). Specifically, it has been shown that both endogenous and exogenous glucocorticoids induce the expression of LDHB and GOT1 in liver of various animal models and that this effect is modulated by multiple promoter elements that are responsive to a variety of physiological stimuli. We believe that the metabolic adaptations to exercise training described in this report are mediated, at least in part, by glucocorticoid receptor activators and coactivators that induce a training-responsive transcriptional response. Interestingly, well-characterized genetic variations in the glucocorticoid receptor have been associated with both visceral adiposity and the metabolic syndrome (14, 48, 36). It is also worth noting that mitochondrial biogenesis is stimulated by glucocorticoids specifically in active skeletal muscle (50), a fact that strengthens our assertion that cortisol may play an important role in muscle metabolic adaptation to exercise. The surprising lack of glucocorticoid-induced expression of GOT1 and LDHB in rat skeletal muscle in response to an exogenous methylprednisolone bolus may reflect tissue-specific or species-specific response to glucocorticoids. It would be worthwhile, therefore, to investigate effects of cortisol on myotubes or in the context of an animal exercise model, because it is known that exercise limits the glucocorticoid-induced muscle atrophy (50). Most studies of glucocorticoid-induced expression of LDHB and GOT1 have been investigated in the liver of nonhuman animal models, and none has yet been conducted in skeletal muscle (41, 42, 46). These studies revealed the presence of NF1 and SPI as well as negative glucose and insulin response elements in close proximity to these GREs, which may function in a combinatorial and tissue-specific manner (7, 37). Chronically high circulating levels of insulin and glucose may, therefore, explain the relatively low expression of GOT1 seen in untrained muscle (Fig. 1), whereas decreased fasting insulin (Fig. 7), and improved glucose tolerance with exercise training may contribute to increased GOT1 expression.

In summary, we propose that changes in glucose metabolism economy that occur in aerobic exercise-trained skeletal muscle are also responsible for the cardiovascular benefits of habitual exercise in individuals with impaired glucose tolerance. We propose also a plausible but unconfirmed model and a plausible mechanism for coordinated regulation of these genes and that these changes are regulated, at least in part, by the (co)activation of the glucocorticoid receptor, which has also been implicated in obesity. Recently, we described significant improvements in muscle-associated changes in circulating fibrinolytic proteins with exercise training (20). Coupled with increased lactate clearance and improved glucose metabolism (22a), microarrays have helped us to describe the molecular basis for the improvement in two cardiovascular risk factors associated with the metabolic syndrome (15) and the importance of skeletal muscle in governing these changes.

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