Alterations in soleus muscle gene expression associated with a metabolic endpoint following exercise training by lean and obese Zucker rats

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Submitted 20 November 2006; accepted in final form 1 February 2007

Ort T, Gerwien R, Lindborg KA, Diehl CJ, Lemieux AM, Eisen A, Henriksen EJ. Alterations in soleus muscle gene expression associated with a metabolic endpoint following exercise training by lean and obese Zucker rats. Physiol Genomics 29: 302–311, 2007. First published February 6, 2007; doi:10.1152/physiolgenomics.00257.2006.—Exercise training decreases insulin resistance and increases glucose tolerance in conditions of prediabetes and overt Type 2 diabetes. However, the adaptive responses in skeletal muscle at the molecular and genetic level for these effects of exercise training have not been clearly established in an animal model of prediabetes. The present study identifies alterations in muscle gene expression that occur with exercise training in prediabetic, insulin-resistant obese Zucker rats and insulin-sensitive lean Zucker rats and are associated with a well-defined metabolic outcome. Treadmill running for up to 4 wk caused significant enhancements of glucose tolerance as assessed by the integrated area under the curve for glucose (AUCg) during an oral glucose tolerance test. Using microarray analysis, we identified a set of only 12 genes as both significantly altered by exercise training (>1.5-fold change; P < 0.05) and significantly correlated (P < 0.05) with the AUCg. Two genes, peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) and protein kinase C-ζ (PKC-ζ), are involved in the regulation of muscle glucose transport, and we provide the first evidence that PGC-1α gene expression is enhanced by exercise training in insulin-resistant muscle. Protein expression of PGC-1α and PKC-ζ were positively correlated with the mRNA expression for these two genes. Overall, we have identified a limited number of genes in soleus muscle of lean and obese Zucker rats that are associated with both decreased insulin resistance and increased glucose tolerance following endurance exercise training. These findings could guide the development of pharmaceutical “exercise mimetics” in the treatment of insulin-resistant, prediabetic, or Type 2 diabetic individuals.

THE METABOLIC SYNDROME, also known as the insulin resistance syndrome, is defined as a clustering of metabolic risk factors in the same individual that includes abdominal obesity, insulin resistance and glucose intolerance, dyslipidemia, and hypertension (20). Although there is much controversy regarding which risk factors should be included as part of the metabolic syndrome (1), it is clear that the aforementioned factors can contribute to the worsening of cardiovascular pathologies and to the development of Type 2 diabetes.

The obese Zucker (fa/fa) rat is a well-established rodent model of prediabetes that displays several characteristics associated with the metabolic syndrome. Hyperphagia develops in this animal due to a point mutation in the leptin receptor gene (18, 27, 31), and the obese Zucker rat consequently develops central obesity, dyslipidemia, extreme hyperinsulinemia, and marked glucose intolerance and insulin resistance (16, 22). However, in the fasting state the obese Zucker rat maintains normal to near-normal glycemia (16).

Endurance exercise training, as well as weight loss, has been shown to decrease the risk factors associated with the metabolic syndrome (reviewed in Ref. 16) and can decrease the conversion from a prediabetic state to overt Type 2 diabetes (11). In the obese Zucker rat, exercise training has been shown to enhance whole body glucose tolerance and to improve insulin-stimulated glucose uptake in skeletal muscle (3, 6, 9, 12, 28, 30). Even 24 h after a bout of exercise, trained skeletal muscle from obese Zucker rats has improved insulin-stimulated glucose uptake compared with muscle from sedentary obese Zucker rats (reviewed in Ref. 16). However, the identification of the adaptive responses in skeletal muscle at the molecular and genetic level responsible for these beneficial effects of exercise training in both humans with prediabetes and the prediabetic obese Zucker rat remains incomplete.

Microarray analysis, a technique for assessing changes in the expression of very large sets of genes in tissues, can facilitate the identification of cellular mechanisms responsible for this increased insulin-stimulated glucose disposal following exercise training by detecting skeletal muscle genes that experience an alteration in their expression due to this intervention. Microarray analysis has been used recently to investigate the effects of exercise training by Zucker Diabetic Fatty (ZDF) rat, a model of overt Type 2 diabetes, on skeletal muscle gene expression (8). The expression of many skeletal muscle genes that encode for glucose and lipid metabolism, as well as for growth factors and various signaling proteins, was upregulated by this intervention (8). However, no study to date has used microarray analysis to assess the adaptive responses of gene expression in skeletal muscle of the prediabetic obese Zucker rat (fa/fa) to exercise training, nor has any previous study been designed to correlate alterations in the expression of these exercise training-sensitive genes with a relevant metabolic outcome, such as glucose tolerance or insulin sensitivity.

In this context, the present investigation was designed to use gene expression profiling to identify genes in type I soleus muscle that respond to endurance exercise training by both normal, insulin-sensitive lean Zucker (Fa/−) rats and insulin-resistant obese Zucker rats. The metabolic relevance of changes in skeletal muscle gene expression due to the exercise training was further assessed by determining the correlation with the changes in an important metabolic outcome: glucose disposal during an oral glucose tolerance test (OGTT). We
hypothesized that there would be a subset of genes in skeletal muscle whose expression would be altered by the exercise training, would be correlated with the expected training-induced improvements in whole body glucose tolerance, and could likely play a causative role in the improved metabolic status.

RESEARCH DESIGN AND METHODS

Animals. Female lean (Fa/−) and obese (fa/la) Zucker rats (Harlan, Indianapolis, IN) were obtained approximately at 5–6 wk of age and separated into sedentary and exercise-trained groups. These groups were then further divided into 1-wk, 2-wk, and 4-wk-treated groups, with four sedentary and four age-matched trained animals in each respective group. Training began 1 wk later. All animals were housed in a temperature-controlled room (20–22°C) with a 12:12-h light-dark cycle at the Central Animal Facility of the University of Arizona. They were given free access to chow (Teklad, Madison, WI) and water. All procedures were approved by the University of Arizona Animal Care and Use Committee.

Exercise training protocol. Animals were exercised on a motorized treadmill for 7 consecutive days during the first week of training and then for 6 days per week for the remainder of the 4-wk training period. The exercise regimen was designed to progressively increase the time the animals ran during the first week (5 min on day 1, 10 min on day 2, 20 min on day 3, etc.). By the end of the first week (day 7), the rats were running a total of 60 min at a speed of 15 m/min and a 4% grade. During the second week, the speed was progressively increased until the animals were running 10 min at 15 m/min, 40 min at 20 m/min, and a final 10 min period at 15 m/min, all at a 4% grade. This protocol was then maintained for the remaining 2 wk of the exercise-training period. We recognize that this moderate-intensity training regimen was more demanding for the obese animals than for the lean animals due to the difference in body weights between the groups, but it did achieve the primary goal of the experimental design, which was to significantly improve glucose tolerance and elicit alterations in muscle gene expression in both lean and obese groups (see RESULTS).

Assessment of oral glucose tolerance. After the desired length of exercise training (1, 2, or 4 wk), animals were food-restricted (4 g of chow for at least 12 h overnight) and were administered an OGTT via a 1 g/kg glucose feeding by gavage. The OGTT was performed 18–24 h after the final bout of exercise. Blood (−0.25 ml) was collected from a cut at the end of the tail immediately before and at 15, 30, 60, and 120 min after glucose administration. Whole blood was then thoroughly vortexed with EDTA (18 mM final concentration) and centrifuged at 13,000 g for 30 s to isolate the plasma. The plasma was then removed and stored at −80°C for glucose (Sigma Chemical, St. Louis, MO) and insulin (Linco Research, St. Charles, MO) assays. Animals were given a 2.5-ml subcutaneous injection of 0.9% saline to compensate for plasma loss during the procedure.

Microarray analysis. The animals returned to the exercise regimen the day after the OGTT and were killed at least 1 day after training had resumed. They were again food-restricted overnight as before, and the next morning at 8 AM were deeply anesthetized with Nembutal (50 mg/kg pentobarbital sodium ip; Abbott Laboratories, North Chicago, IL). Both soleus muscles were quickly removed, immediately frozen in liquid nitrogen, and stored at −80°C until analysis. The muscle isolation, like the OGTT, was performed 18–24 h after the final bout of exercise. This interval between the last bout of exercise and sample preparation ensures that the changes identified represent stable, adaptive responses to the exercise training rather than acute alterations in gene expression due to the last bout of exercise. The soleus muscle, which consists of primarily type I fibers (slow-twitch oxidative), was chosen for analysis because it is highly responsive to exercise training regimen in lean and obese Zucker rats (3, 6, 12, 28, 30).

Microarray analysis on the soleus muscle was completed by Curagen. Samples were hybridized on the Whole Rat Genome CodeLink Microarray Chip (~35,000 oligos corresponding to rat transcripts and expressed sequence tags (ESTs)). Hybridization was performed by purifying mRNA from the tissue samples and assessing its quantity and quality through an Agilent Bioanalyzer and spectrophotometry. cDNA was prepared from the total RNA and biotinylated cRNA was prepared from the DNA template using standard methods (10, 21). The cRNA was then verified on the Agilent Bioanalyzer and fragmented to uniform size (also verified on the bioanalyzer). The Whole Rat Genome CodeLink Micorarray Chip was then hybridized with the fragmented cRNA and stained with Cy5-streptavidin. Slides were then washed and scanned on an Axon GenePix Scanner. Data were log2 transformed and normalized to the 75th percentile of all arrays. Features whose mean value was below the median threshold for the arrays and features that were saturated were removed from further consideration (~23,000). The gene array data for the soleus in this study are deposited in ArrayExpress (accession number E-TABM-219).

Protein expression of PGC-1α, PKC-ζ, and GLUT-4. The protein expression levels of peroxisome proliferator-activated receptor-γ co-activator 1α (PGC-1α), the ζ-isoform of protein kinase C (PKC-ζ), and glucose transporter (GLUT)-4 glucose transporter in soleus muscle were determined. A section of soleus was removed from the whole frozen muscle on dry ice. The sectioned muscle was then homogenized with 8 volumes of ice-cold homogenization buffer consisting of 50 mM HEPES (pH 7.4), 150 mM NaCl, 20 mM Na pyrophosphate, 20 mM β-glycerophosphate, 10 mM NaF, 2.0 mM Na3VO4, 2.0 mM EDTA (pH 8.0), 1% Triton X-100, 10% glycerol, 1.0 mM MgCl2, 10 mM CaCl2, aprotinin (10 μM/L), leupeptin (10 μM/L), pepstatin, PMSF (2 mM), and incubated on ice for 20 min. The homogenate was then centrifuged for 20 min at 13,000 g at 4°C. The resultant supernatant was removed and stored at −80°C. Total protein was determined using the bicinchoninic acid method (Sigma). The homogenates where then aliquotted into sample total protein concentrations of 250 μg/35 μL, combined with 3× Laemmli sample buffer and PBS, boiled for 5 min, and stored at −80°C.

Samples of equal total protein content were loaded on 7.5% polyacrylamide gels (Bio-Rad Laboratories, Hercules, CA), separated by SDS-PAGE, and then transferred to nitrocellulose membranes. The nitrocellulose membranes where rinsed in ddH2O and blocked for 1 h in 5% PBS-T (0.05% Tween)-milk solution, and then rinsed in PBS-T and finally in PBS. Thereafter, membranes were incubated with primary antibody for either PGC-1α (Santa Cruz) at a 1:1,000 concentration, PKC-ζ (AbCam, Cambridge, MA), or GLUT-4 (MorphoSys, Kingston, NH) at a 1:250 concentration overnight with constant agitation at 4°C. The membranes were then washed twice for 10 min in PBS-T and twice for 5 min in PBS and finally incubated with a secondary antibody, goat anti-rabbit conjugated with horseradish peroxidase (Chemicon, Temecula, CA). The proteins were visualized by an enhanced chemiluminescence detection system (Amersham Pharmacia, Piscataway, NJ) on Kodak X-Ormat AR film (Kodak, Rochester, NY). The band intensities were quantified on a scanning densitometer (Bio-Rad Model GS-800) with Quantity One Software (Bio-Rad).

Statistical analysis. Data are presented as means ± SE for four animals per group. Expression data were analyzed in two ways. A three-way analysis of variance was used to identify genes modulated by exercise (other effects included time and rat strain). Genes with a strong signal (P < 0.05) were subsequently filtered based on fold-change and overall expression. Gene expression differences had to reach a minimum threshold of a 1.5-fold difference between any two subsets and had to have a baseline expression above threshold on average across the treatment groups. The second analysis involved correlation of the integrated glucose area under the curve (AUCg) with gene expression across all genes. Significant associations were identified (P < 0.05) and subsequently filtered by removing those
with an expression level below threshold, on average. We did not control for multiplicity in identifying associations, as microarray experiments are traditionally underpowered. Instead, we restricted our hypothesis to the simultaneous occurrence of two events. Statistical analyses were performed using the R software system, version 2.0.1.

RESULTS

Effect of exercise training on glucose tolerance and insulin sensitivity. The exercise training did not significantly affect the final body weights of the animals in either the lean or obese groups (4-wk lean sedentary = 178 ± 4 g; 4-wk lean trained = 181 ± 8 g; 4-wk obese sedentary = 298 ± 31 g; 4-wk obese trained = 302 ± 8 g).

The fasting glucose levels of the sedentary obese Zucker rats were comparable to those of the sedentary lean Zucker rats over the 4-wk experimental period (Fig. 1, top). The exercise training caused a significant $P < 0.05$ decrease in fasting plasma glucose only in the 4-wk obese animals. While the exercise regimen had no effect on glucose tolerance after the first week in either the obese or lean groups, by week 2 the exercise-trained obese group showed a significant 10% decrease in the integrated AUCg (Table 1). At 4 wk, the exercise-trained obese group continued to display an increased glucose tolerance (18% decrease in AUCg), and the exercise-trained lean group also displayed a significant 10% decrease in AUCg.

The effect of the exercise-training regimen on the insulin response (Fig. 2, top) and on integrated area under the curve for insulin (AUCi) during the OGTT (Fig. 2, bottom) was also determined. In the lean groups, there were no significant differences detected for AUCi between trained and sedentary animals. In the obese animals, there was a significant reduction in the AUCi only in the 4-wk exercise-trained group.

Exercise training did have a significant effect on the plasma-insulin index, an indicator of whole body insulin sensitivity defined as the product of the AUCg and the AUCi (9) (Fig. 3). In the lean animals, there was a trend toward a decreased glucose-insulin index (indicating increased insulin sensitivity) after 2 wk of training, and this parameter was significantly reduced ($22\%, \ P < 0.05$) after 4 wk of training. The obese animals experienced significant reductions in the glucose-insulin index after both 2 wk (19%) and 4 wk (31%) of training.

Effect of exercise training on skeletal muscle gene transcription. Microarray analysis revealed that in soleus muscle 34 probes were significantly upregulated or downregulated (>1.5-fold change in gene transcription; $P < 0.05$) due to exercise training, and 1,565 probes were significantly correlated ($P < 0.05$) with OGTT AUCg (encompassing differences due to both obesity and training status). Fourteen probes corresponding to 12 genes (two genes were represented by two sets of probes) were common for both lists, e.g., their expression had been altered by exercise training, and these exercise-training-induced differences in gene expression were correlated with the AUCg (Table 1). It should be noted that various well-known "diabetes genes" were modulated by the exercise training but may have been altered only in one of the two phenotypes. For example, GLUT-4 mRNA was elevated 1.5-fold ($P = 0.0010$) due to the exercise training, but only in soleus muscle from the obese Zucker rats, and was significantly correlated with the alterations in glucose tolerance in these obese animals ($P = 0.0088$). Interestingly, there was no alteration in soleus GLUT-4 protein levels after 1 wk of training in either lean or obese groups, but after 2 wk there were trends ($P < 0.10$) for increased GLUT-4 in the lean (100 ± 7 relative units in the sedentary group vs. 115 ± 2 in the trained group) and obese groups (100 ± 6 vs. 120 ± 2), and after 4 wk there was a trend for an increase ($P < 0.10$) in the lean group (100 ± 11 vs. 125 ± 6) and a significant 41% increase ($P < 0.05$) in the obese group (100 ± 10 vs. 141 ± 15).

We focused on two of the exercise training-inducible genes, PGC-1α and PKC-ζ, as PKC-ζ is specifically involved in the insulin signaling pathway (17, 23) and PGC-1α participates in the adaptive responses to exercise training for expression of glucose transporters and mitochondrial enzymes in skeletal muscle (reviewed in Ref. 34). PGC-1α mRNA was upregulated more than twofold and PKC-ζ mRNA was upregulated ~1.7-fold in exercise-trained soleus muscle (Table 1). To test whether the exercise training-induced changes in mRNA levels for these two specific genes were correlated with the alterations in AUCg due to training, we performed linear regression analysis comparing the mRNA expression levels and AUCg values from each individual animal. For both PGC-1α (Fig. 4, left) and PKC-ζ (Fig. 4, right), highly significant negative correlations were detected.

Effects of exercise training on PGC-1α and PKC-ζ protein expression. Since mRNA levels of PGC-1α and PKC-ζ were both upregulated with exercise training and were negatively correlated with the AUCg, the protein levels of these genes in soleus muscle were measured to verify the complete gene translation. For PGC-1α in muscle from the lean animals, there were trends toward increased protein expression levels after 1 wk and 4 wk of training, and a significantly ($P = 0.032$) increased PGC-1α protein expression level was observed in the 2-wk-trained lean group (Fig. 5, top left). For the obese animals, PGC-1α protein expression was significantly increased with exercise training at all time points (34% at week 1, 33% at week 2, and 41% at week 4) (Fig. 5, top right).

PKC-ζ protein expression was also increased with exercise training in both lean and obese animals. Both lean and obese groups showed a trend toward an increase in PKC-ζ protein expression after 1 and 2 wk of training (Fig. 5, bottom left and bottom right, respectively). Moreover, significant increases in soleus PKC-ζ protein expression were detected in the 4-wk-trained lean animals (59%) and 4-wk-trained obese animals (39%).

Correlations between PGC-1α and PKC-ζ protein expression and glucose tolerance. As already shown in Fig. 4, the mRNA expression levels for both PGC-1α and PKC-ζ were significantly and negatively correlated with the AUCg. The protein expression levels of these factors were also negatively correlated with the AUCg (Fig. 6). The correlation between PGC-1α protein and the AUCg (Fig. 6, left) approached statistical significance ($P = 0.072$), whereas that between PKC-ζ protein expression and the AUCg (Fig. 6, right) was significant ($P = 0.032$).

Correlations between PGC-1α and PKC-ζ protein expression and mRNA expression. Since the mRNA and protein levels individually were negatively correlated with the AUCg, the relationship between the exercise training-induced upregulation of mRNA and the protein expression for PGC-1α and PKC-ζ was then assessed by linear regression. The protein expression for both PGC-1α (Fig. 7, left) and PKC-ζ (Fig. 7,
Fig. 1. Effects of exercise training on the glucose response during an oral glucose tolerance test (OGTT) in lean and obese Zucker rats. Mean values ± SE for glucose during the OGTT are shown at left, with corresponding integrated glucose areas under the curve (AUC) shown at right. No error bars are shown when the symbol is larger than the SE value. Values are means for 4 animals per group. *P < 0.05, trained vs. sedentary.
Fig. 2. Effects of exercise training on the insulin response during an OGTT in lean and obese Zucker rats. Mean values ± SE for insulin during the OGTT are shown at left, with corresponding insulin AUCs shown at right. No error bars are shown when the symbol is larger than the SE value. Values are means for 4 animals per group. */P < 0.05, trained vs. sedentary.
were positively correlated with their respective mRNA expression levels, indicating a functional significance of the enhanced mRNA expression for these two important metabolic genes.

**DISCUSSION**

In the present study, microarray analysis was used to identify numerous genes in type I skeletal muscle of both insulin-sensitive female lean Zucker rats and prediabetic, insulin-resistant female obese Zucker rats that are responsive to endurance exercise training (Table 1). Many of the protein products of these genes are involved in important signaling pathways and in carbohydrate and lipid metabolism and serve as transcription factors within the cell. Moreover, this is the first study to correlate these exercise training-induced alterations in gene transcription with a relevant metabolic outcome, namely the AUCg during an OGTT, which is a well-accepted index of glucose tolerance. This correlation analysis identified a limited set of exercise training-inducible genes that are associated with the overall improvements in glucose disposal.

![Fig. 3. Effect of exercise training on the glucose-insulin index in lean and obese Zucker rats. Values are means ± SE for 4 animals per group. *P < 0.05, trained vs. sedentary.](http://physiolgenomics.physiology.org/)

**Table 1. Genes in soleus muscle of Zucker rats both significantly affected by exercise training and significantly correlated with the exercise-induced alterations in glucose tolerance**

<table>
<thead>
<tr>
<th>Gene Description</th>
<th>Gene ID</th>
<th>Pathway</th>
<th>Log2 (Mean Intensity)</th>
<th>Fold-Difference in Trained vs. Sedentary groups</th>
<th>P</th>
<th>Correlation with AUCg</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prkcz: protein kinase C, zeta</td>
<td>NM_022507.1</td>
<td>signaling</td>
<td>5.166</td>
<td>5.938</td>
<td>1.71</td>
<td>&lt;0.0001</td>
<td>-0.55</td>
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<td>Mybph: myosin binding protein H</td>
<td>NM_031813.1</td>
<td>structural</td>
<td>9.192</td>
<td>9.789</td>
<td>1.51</td>
<td>0.0067</td>
<td>-0.33</td>
</tr>
<tr>
<td>CPLX1: complexin 1</td>
<td>BI281681.1</td>
<td>trafficking</td>
<td>6.088</td>
<td>6.834</td>
<td>1.68</td>
<td>&lt;0.0001</td>
<td>-0.36</td>
</tr>
<tr>
<td>CPLX2: complexin 2</td>
<td>BF544703.1</td>
<td>trafficking</td>
<td>5.965</td>
<td>7.259</td>
<td>2.45</td>
<td>&lt;0.0001</td>
<td>-0.43</td>
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<tr>
<td>Ppargc1: peroxisome proliferative activated receptor, gamma, coactivator 1</td>
<td>NM_031347.1</td>
<td>transcription</td>
<td>8.672</td>
<td>9.771</td>
<td>2.14</td>
<td>&lt;0.0001</td>
<td>-0.46</td>
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<td>Zfp36: zinc finger protein 36</td>
<td>NM_133290.2</td>
<td>transcription</td>
<td>8.185</td>
<td>7.515</td>
<td>-1.59</td>
<td>&lt;0.0001</td>
<td>0.31</td>
</tr>
<tr>
<td>FOS: v-fos FBJ murine osteosarcoma viral oncogene homolog</td>
<td>BF415939.1</td>
<td>transcription</td>
<td>8.948</td>
<td>7.935</td>
<td>-2.02</td>
<td>&lt;0.0001</td>
<td>0.40</td>
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<tr>
<td>FOS: v-fos FBJ murine osteosarcoma viral oncogene homolog</td>
<td>AW915240.1</td>
<td>transcription</td>
<td>7.819</td>
<td>7.189</td>
<td>-1.55</td>
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<tr>
<td>LG12: leucine-rich repeat LGI family, member 2</td>
<td>BI295858.1</td>
<td>other</td>
<td>5.978</td>
<td>6.567</td>
<td>1.50</td>
<td>&lt;0.0001</td>
<td>-0.30</td>
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<td>HSF2BP: heat shock transcription factor 2 binding protein</td>
<td>BE106403.1</td>
<td>other</td>
<td>6.161</td>
<td>6.830</td>
<td>1.59</td>
<td>&lt;0.0001</td>
<td>-0.32</td>
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<tr>
<td>GSR: glutathione reductase Similar to OTU domain containing 1 (predicted)</td>
<td>BE106403.1</td>
<td>other</td>
<td>6.161</td>
<td>6.830</td>
<td>1.59</td>
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Data are for 12 animals in each sedentary and trained group. AUCg, glucose area under the curve.
that develop with a well-tolerated endurance training program in Zucker rats.

The alterations in endurance exercise-related gene transcription in the obese Zucker rats have also been studied in male ZDF rats (8). The male ZDF rat is a model of overt Type 2 diabetes, characterized by obesity and a highly predictable conversion from an initial prediabetic state to a condition of Type 2 diabetes (13, 32), but is a model distinct from the female obese Zucker rat. Colombo et al. (8) found that the expression of 302 genes increased and the expression of 119 genes decreased after endurance training in Zucker rats.

**Fig. 4.** Correlation analysis between PGC-1α and PKC-ζ mRNA and the glucose AUC (AUCg). The correlation between the AUCg and the mRNA expression of peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α (left) and PKC-ζ (right) was performed by linear regression analysis. Data are from soleus muscle from sedentary lean (●), sedentary obese (○), trained lean (■), and trained obese (●) Zucker rats.

**Fig. 5.** Effect of exercise training on the protein expression of PGC-1α and PKC-ζ in soleus muscle of lean and obese Zucker rats. PGC-1α protein was determined in soleus muscle after 1, 2, or 4 wk of exercise training in soleus muscle of lean (top left) and obese (top right) Zucker rats. Likewise, protein expression of PKC-ζ in soleus muscle from lean (bottom left) and obese (bottom right) was determined after these periods of exercise training. Cross-hatched bars represent sedentary animals, and filled bars represent trained animals. Representative bands from the autoradiographs are presented above the bars. Values are means ± SE for 4 animals per group. *P < 0.05, trained vs. sedentary.
genes decreased following 5 wk of endurance exercise training by male ZDF rats on a motorized treadmill. In the present investigation using female prediabetic obese Zucker rats, substantially fewer genes were altered following up to 4 wk of exercise training (Table 1). It should be noted, however, that the ZDF rats involved in the study of Colombo et al. (8) displayed more severe metabolic derangements than the obese Zucker rats in the present study. However, both studies did identify alterations in numerous genes encoding for proteins involved in glucose metabolism and signaling pathways (Table 1 and Ref. 8). What distinguishes the present study from that of Colombo and colleagues (8) is the additional correlation of gene expression to a measure of the metabolic outcome in all the animals: the improved AUCg associated with exercise
training. In this fashion we were able to focus attention on a smaller set of genes (12 vs. 421) with a potentially central role in the improved physiological state. This suited our goal of identifying a tractable number of intervention points for the development of “exercise mimetic” pharmaceuticals that improve AUCg.

Two of the genes (PGC-1α and PKC-ζ) that were significantly altered with exercise training were also found to have a significant increase in protein expression in the skeletal muscle of the lean and obese Zucker rats. PGC-1α was upregulated in both lean and obese animals with exercise training (Fig. 5, top), and PKC-ζ was also upregulated significantly in both lean and obese animals after 4 wk of training (Fig. 5, bottom). PGC-1α is a ligand that binds to transcription factors to form a protein complex that promotes the unwinding of the DNA strand and binding of RNA polymerase II to initiate transcription (24).

PGC-1α gene transcription can be upregulated via input from several intracellular pathways, including those utilizing the calcium/calmodulin-dependent protein kinase (33), calcineurin, p38 mitogen-activated protein kinase (14), and the 5′-AMP-activated protein kinase (19). PGC-1α is also involved in the upregulation of muscle glucose metabolism through partnering with the myocyte enhancer factor-2 to increase GLUT-4 expression levels (reviewed in Ref. 26). PGC-1α protein upregulation has been seen in skeletal muscle of humans and rats that have undergone endurance training (14). We have also shown this exercise training-induced protein upregulation (Fig. 5), as well as an increase in mRNA expression (Table 1). Interestingly, we did find an increase in GLUT-4 mRNA and protein expression with the exercise training, but these effects were statistically significant only in soleus muscle of the obese Zucker rats. The lack of a significant effect in the lean rats may be attributed to the 24-h time period between the last bout of exercise and tissue collection and to the relatively mild exercise intervention in these lean animals. GLUT-4 mRNA and protein may have been upregulated immediately postexercise in lean soleus but may have decreased during the intervening 24-h period before muscle collection. It should be noted that numerous studies using both lean and obese Zucker rats have demonstrated exercise training-induced increases in skeletal muscle GLUT-4 protein expression (3, 6, 12, 15, 28–30) 24–48 h after the final training session.

This is the first study to identify an increase in PKC-ζ gene and protein expression in skeletal muscle of an obesity-associated animal model of insulin resistance due to exercise training (Table 1, Fig. 5), and the first to correlate these exercise training-induced alterations in PKC-ζ expression with a metabolic outcome (AUCg) in these animals (Figs. 4 and 6). PKC-ζ has been found to positively affect GLUT-4-mediated glucose transport in insulin-sensitive skeletal muscle (25, 34), and the activation of PKC-ζ is enhanced by an acute bout of exercise in human skeletal muscle (26). Moreover, insulin-regulated activation of PKC-ζ is impaired in insulin-resistant human skeletal muscle (4). However, in contrast to the present investigation in lean Zucker rats, chronic exercise training did not alter PKC-ζ protein expression in skeletal muscle of insulin-sensitive Sprague-Dawley rats (5) or humans (24).

PKC-ζ can be stimulated by two different pathways: 1) the insulin-signaling pathway, in which PKC-ζ is activated by phosphatidylinositol-3-kinase (PI-3 kinase) along with Akt (25, 34), and 2) in the extracellular regulated kinase pathway, in which phospholipase D is activated and generates phosphatidic acid to directly activate PKC-ζ (7). PI-3 kinase mRNA was also found to be upregulated in soleus muscle of the 2- and 4-wk trained obese animals, although there was no change in mRNA expression for other insulin signaling factors (data not shown). Collectively, these data support an important role of PKC-ζ in the increased glucose tolerance found in the exercised animals, perhaps by stimulating GLUT-4 translocation to the cellular membrane and allowing a greater rate of glucose transport in response to an insulin stimulus. It has been shown previously by our research group that 2–6 wk of endurance exercise training increases insulin-stimulated glucose uptake in the soleus muscle from lean Zucker rats (15) and obese Zucker rats (28, 29, 31). The present data may also reflect a situation where the exercise training-induced upregulation of a distal element of the insulin signaling cascade, PKC-ζ, may overcome defects in the expression and functionality of upstream insulin signaling factors associated with the insulin-resistant state.

One long-term goal of this avenue of investigation is to find target genes, modulated by exercise training and associated with improvements in skeletal muscle and whole body insulin action, that could be similarly modulated by a pharmaceutical intervention to increase glucose tolerance and insulin sensitivity in conditions of insulin resistance, such as prediabetes and Type 2 diabetes. From our present study, PGC-1α and PKC-ζ, as well as several other gene products, seem to be factors that could be targeted to help increase insulin action on skeletal muscle glucose uptake and their regulation should be the topic of future investigations.

In summary, using microarray analysis, we have demonstrated in insulin-sensitive lean Zucker rats and in markedly insulin-resistant obese Zucker rats that a small subset of alterations in soleus muscle gene expression was significantly correlated with increased whole body glucose tolerance following endurance exercise training. Two of these muscle genes, PGC-1α and PKC-ζ, displayed significantly enhanced protein expression following exercise training in the lean and obese Zucker rats, and these protein products appear to be appropriate candidates for future investigation of factors that improve skeletal muscle glucose uptake and whole body glucose tolerance in insulin-resistant individuals. PGC-1α and PKC-ζ, as well as several other proteins derived from genes identified as exercise training inducible, should be viewed as entities that identify intracellular pathways harboring pharmacological targets that can improve whole body glucose tolerance and insulin sensitivity in insulin-resistant conditions.

ACKNOWLEDGMENTS

The authors thank Mary K. Teachey and Julie A. Sloniger for excellent technical assistance.

GRANTS

This study was supported by a grant from CuraGen Corporation (to E. J. Henriksen) and by National Institute of Diabetes Digestive and Kidney Diseases Grant DK-063967 (to E. J. Henriksen).

REFERENCES

GENE EXPRESSION AND EXERCISE TRAINING


