Interactions of exercise training and ACE inhibition on insulin action in obese Zucker rats

MICHELLE S. STEEN, KARA R. FOIANINI, ERIK B. YOUNGBLOOD, TYSON R. KINNICK, STEPHAN JACOB, AND ERIK J. HENRIKSEN
Muscle Metabolism Laboratory, Department of Physiology, University of Arizona, Tucson, Arizona 85721-0093

Steen, Michelle S., Kara R. Foianini, Erik B. Youngblood, Tyson R. Kinnick, Stephan Jacob, and Erik J. Henriksen. Interactions of exercise training and ACE inhibition on insulin action in obese Zucker rats. J. Appl. Physiol. 86(6): 2044–2051, 1999.—Exercise training or chronic treatment with angiotensin-converting enzyme (ACE) inhibitors can ameliorate glucose intolerance, insulin resistance of muscle glucose metabolism, and dyslipidemia associated with the obese Zucker rat. The purpose of the present study was to determine the interactions of exercise training and ACE inhibition (trandolapril) on these parameters in the obese Zucker rat. Animals were assigned to a sedentary control, a trandolapril-treated (1 mg·kg⁻¹·day⁻¹ for 6 wk), an exercise-trained (treadmill running for 6 wk), or a combined trandolapril-treated and exercise-trained group. Exercise training, alone or with trandolapril, significantly (P < 0.05) increased peak O₂ consumption by 31–34%. Similar decreases in fasting plasma insulin (34%) and free fatty acids (31%) occurred with exercise training alone or in combination with trandolapril. Compared with control, exercise training or trandolapril alone caused smaller areas under the curve (AUC) for glucose (12–14%) and insulin (28–33%) during an oral glucose tolerance test. The largest decreases in the glucose AUC (40%) and insulin AUC (53%) were observed in the combined group. Similarly, whereas exercise training or trandolapril alone improved maximally activated insulin-stimulated glucose transport in isolated epitrochleis (26–34%) or soleus (39–41%) muscles, the greatest improvements in insulin action (67 and 107%, respectively) were seen in the combined group and were associated with similarly enhanced muscle GLUT-4 protein and total hexokinase levels. In conclusion, these results indicate combined exercise training and ACE inhibition improve oral glucose tolerance and insulin-stimulated muscle glucose transport to a greater extent than does either intervention alone.

insulin resistance; trandolapril; glucose tolerance; muscle glucose transport; GLUT-4 protein; angiotensin-converting enzyme

The insulin resistance syndrome is characterized by the clustering of several atherogenic risk factors in the same individual, including essential hypertension, glucose intolerance, insulin resistance of skeletal muscle glucose disposal, hyperinsulinemia, dyslipidemia, and central obesity (11). Two important factors involved in the development of this condition are skeletal muscle insulin resistance and the accompanying hyperinsulinemia (11, 24, 27), both of which are themselves cardiovascular disease risk factors (21). Because >50% of hypertensive individuals are insulin resistant and hyperinsulinemic (24), a prudent course of action in treating individuals with the insulin resistance syndrome would be the use of interventions that lower blood pressure as well as reduce insulin resistance and improve glucose tolerance.

Two such interventions are aerobic exercise training and chronic treatment with angiotensin-converting enzyme (ACE) inhibitors. Endurance exercise training by previously sedentary glucose-intolerant and insulin-resistant humans results in enhanced insulin action on skeletal muscle glucose disposal that is associated with a substantial increase in muscle GLUT-4 protein level, suggesting a potential cause-and-effect relationship (18). In addition, moderate and high-intensity aerobic training by the obese Zucker rat, a widely used animal model of obesity-related glucose intolerance, insulin resistance, and dyslipidemia, increase glucose tolerance and glucose disposal (3, 9), primarily because of adaptations in the skeletal muscle glucose transport process (9, 19, 37), including upregulation of GLUT-4 protein levels (2, 6, 13) and increased incorporation of GLUT-4 protein into the sarcolemmal membrane (13).

Several clinical investigations have shown that treatment with ACE inhibitors improves insulin sensitivity in hypertensive and insulin-resistant subjects (22–25, 29, 30, 33, 34, 36). In addition, chronic treatment with the ACE inhibitors captopril or trandolapril enhances glucose tolerance and skeletal muscle insulin-mediated glucose transport activity in the insulin-resistant obese Zucker rat (10, 16, 20).

Although the individual effects of aerobic exercise training and ACE inhibition on insulin action are well documented, the metabolic consequences of combining these two interventions in a model of insulin resistance are presently unknown. In this context, the present study was undertaken to determine whether 6 wk of exercise training and 6 wk of administration of the ACE inhibitor trandolapril, in combination, could improve the glucose intolerance, insulin resistance of muscle glucose metabolism, and dyslipidemia (as reflected by plasma free fatty acid levels) associated with the obese Zucker rat to a greater degree than either intervention used individually. Furthermore, we investigated whether the changes in skeletal muscle insulin action brought about by these interventions were associated with alterations in the muscle level of GLUT-4 protein and enzymes involved in glucose phosphorylation (total hexokinase activity) and glucose oxidation (citrate synthase activity).
METHODS

Animals. Female obese Zucker (fa/fa) rats were received from Harlan (Indianapolis, IN) at ~5 wk of age, weighing 150-160 g. The animals were housed in a temperature-controlled room (20–22°C) with a reversed 12:12-h light-dark cycle (lights on from 7 PM to 7 AM) at the Central Animal Facility of the University of Arizona. The animals had free access to chow (Purina, St. Louis, MO) and water. All procedures were approved by the University of Arizona Animal Use and Care Committee.

The rats were randomly assigned to one of the following groups: sedentary control; exercise-trained; trandolapril-treated; or combined trandolapril-treated and exercise-trained. Animals in the trandolapril-treated groups were administered trandolapril (1 mg/kg body wt) by gavage every evening for 6 wk. Animals in the exercise-trained groups were run on a 10-lane motorized rodent treadmill for 6 wk. During the first 2 wk of training, the animals ran every day, and the training protocol was quickly increased to 60 min/day at 4% grade, continuously rotating through the following 15-min cycles: 24 m/min for 10 min, 26 m/min for 3 min, and 28 m/min for 2 min. During the final 4 wk of training, the animals ran 5 days/wk for 90 min/day at 4–8% grade. After this latter training period, the animals continuously rotated through 15-min cycles of running at 26 m/min for 10 min, 30 m/min for 3 min, and 24 m/min for 2 min, all at 4–8% grade.

Oral glucose tolerance tests (OGTTs). After 6 wk, an OGTT was performed in each animal. The rats were restricted to 4 g of chow after 6 PM of the evening before the test. Between 8 and 9 AM on the day of the OGTT, ~12 h after the last trandolapril treatment and/or 24 h after the last exercise bout, the rats were administered a 1 g/kg body weight glucose load by gavage (9). Blood was collected from a small cut at the tip of the tail immediately before and at 15, 30, and 60 min after glucose administration, thoroughly mixed with EDTA (final concentration of 18 mg/ml), and centrifuged at 13,000 g to isolate the plasma. The plasma was stored at ~80°C and subsequently assayed for glucose (Sigma Chemical, St. Louis, MO), insulin by radioimmunoassay (Linco, St. Louis, MO), and free fatty acids (Wako, Richmond, VA). After the final blood collection, each animal was given 2 ml of sterile saline subcutaneously to account for plasma volume loss. On completion of the OGTTs, animals in the exercise training groups were run for 30 min. No animals were treated with trandolapril on this day.

Peak $\text{O}_2$ consumption ($V_{\text{O}_2\text{peak}}$). Aerobic capacity was assessed in each animal by the $V_{\text{O}_2\text{peak}}$ during a treadmill test 48 h after the OGTT, using the method of Bedford et al. (4). No exercise was performed on the day before the $V_{\text{O}_2\text{peak}}$ test; however, trandolapril was administered to the trandolapril group and the combined exercise-trained and trandolapril-treated group. Animals were run on a motorized treadmill in an airtight Plexiglas chamber. Grade and speed of the treadmill were increased every 3 min from a basal level of 0% grade and 13.4 m/min through the following stages: 16.1 m/min at 5%, 21.4 m/min at 10%, 26.8 m/min at 10%, 32.2 m/min at 12%, 32.2 m/min at 15%, 32.2 m/min at 18%, and 32.2 m/min at 21%. The test was terminated when the rats were unable to keep pace with the treadmill belt. $O_2$ (Ametek S-3A1, Applied Electrochemistry, Pittsburgh, PA) and $CO_2$ (Ametek CD-3A) were measured in expired gases every 3 min for the determination of oxygen uptake (ml $O_2$/kg body wt $^{-1}$·min $^{-1}$). Exercise training and trandolapril treatments were resumed for 1 day after the $V_{\text{O}_2\text{peak}}$ assessment.

Muscle glucose transport activity. Approximately 72 h after the $V_{\text{O}_2\text{peak}}$ test and 24 h after the final exercise bout and/or trandolapril treatment, animals were weighed and deeply anesthetized by using an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt). The determination of muscle glucose transport activity, assessed by using 2-deoxyglucose (2-DG) uptake, was started at 8 AM after an overnight food restriction, as described above. One soleus and both epitrochlearis muscles were dissected and prepared for in vitro incubation. Whereas the epitrochlearis muscles were incubated intact, the soleus muscle was prepared into two strips (~25 mg) and incubated. Each muscle was incubated for 1 h at 37°C in 3 ml oxygenated (95% $O_2$-5% $CO_2$) Krebs-Henseleit buffer (KHB) supplemented with 8 mM glucose, 32 mM mannitol, and 0.1% BSA (radioimmunoassay grade, Sigma Chemical). One epitrochlearis muscle and one soleus strip were incubated in the absence of insulin, and the contralateral epitrochlearis muscle and soleus strip were incubated in the presence of a maximally effective concentration of insulin (2 μU/ml; Humulin, Eli Lilly, Indianapolis, IN).

After this initial incubation period, the muscles were rinsed for 10 min at 37°C in 3 ml oxygenated KHB containing 40 mM mannitol, 0.1% BSA, and insulin, if previously present. Thereafter, the muscles were transferred to 2 ml KHB, containing 1 mM 2-[1,2-$^3H$]DG (300 μCi/mmol; Sigma Chemical), 39 mM [U-$^14C$]mannitol (0.8 μCi/mmol; ICN Radiochemicals, Irvine, CA), 0.1% BSA, and insulin, if present previously. At the end of this final 20-min incubation period at 37°C, the muscles were removed, trimmed of excess fat and connective tissue, quickly frozen between aluminum blocks cooled in liquid nitrogen, and weighed. The epitrochlearis muscles were divided into two pieces, which were individually reweighed. One piece from each epitrochlearis muscle and the entire soleus strip were dissolved in 0.5 ml 0.5 N NaOH. After the muscles were completely solubilized, 5 ml of scintillation cocktail was added, and the specific intracellular accumulation of 2-DG was determined as described previously (15). This method for assessing glucose transport activity in isolated muscle has been validated (14).

Biochemical assays. The remaining two pieces of epitrochlearis were pooled, reweighed, and homogenized in 40 volumes of ice-cold 20 mM HEPES (pH 7.4) containing 1 mM EDTA and 250 mM sucrose. These homogenates were used for determination of total protein content by using the bicinchoninic acid method (Sigma Chemical), GLUT-4 protein level (15), total hexokinase activity (35), and citrate synthase activity (31). In addition, the contralateral soleus and plantaris muscles and the heart were removed, trimmed of fat and excess connective tissue, quickly frozen in liquid nitrogen, weighed, and used for subsequent determination of these same variables.

Statistical analysis. All data are presented as means ± SE. The significance of differences among groups was assessed by a factorial ANOVA with a post hoc Fisher’s protected least significant difference test (StatView version 5.0, SAS Institute, Cary, NC). A P value of <0.05 was considered statistically significant.

RESULTS

Body weights, muscle weights, and $V_{\text{O}_2\text{peak}}$. Final average body weights for the trandolapril-treated, exercise-trained, and combined exercise-trained and trandolapril-treated groups were significantly (P < 0.05) lower than those of the sedentary control group (Table 1). There were no significant differences among the groups for average wet weights of the whole epitrochlearis, soleus, or plantaris muscles (data not shown).
Table 1. Final body weights and heart wet weights in obese Zucker rats after the 6-wk intervention periods

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Wt, g</th>
<th>Heart Wt, mg</th>
<th>Heart Wt Wt-to-Body Wt Ratio, mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obese sedentary</td>
<td>362 ± 5</td>
<td>732 ± 14</td>
<td>2.02 ± 0.03</td>
</tr>
<tr>
<td>Obese trandolapril-treated</td>
<td>327 ± 3</td>
<td>597 ± 6</td>
<td>1.83 ± 0.02</td>
</tr>
<tr>
<td>Obese exercise-trained</td>
<td>330 ± 4*</td>
<td>788 ± 25‡</td>
<td>2.38 ± 0.07‡</td>
</tr>
<tr>
<td>Obese combined</td>
<td>322 ± 8*</td>
<td>727 ± 17‡</td>
<td>2.26 ± 0.04‡</td>
</tr>
</tbody>
</table>

Values are means ± SE for 5–9 animals/group. *P < 0.05 vs. obese sedentary group, †P < 0.05 vs. obese trandolapril group. ‡P < 0.05 vs. obese exercise-trained group.

Trandolapril treatment alone induced a significant reduction in heart mass relative to the sedentary control group, both in absolute (18%) and relative (9%) terms, consistent with its known effects on regression of myocardial mass in the obese Zucker rat (20). Both absolute (8%) and relative (18%) heart wet weights in the exercise-trained group were significantly larger than in the sedentary control group. Trandolapril treatment of the exercise-trained obese animals did not prevent this apparent training-induced hypertrophy of the myocardium relative to body weight (12% larger than sedentary control).

As shown in Table 2, the 6-wk period of exercise training resulted in significantly greater peak aerobic capacities in both the exercise-training-only group (34%) and the combined exercise training and trandolapril group (31%) compared with the sedentary control group. Moreover, the exercise-training-only group ran significantly longer (29%) than the sedentary control group during the \( \text{V} \text{O}_{2\text{peak}} \) test, with the greatest improvement in run time (51%) displayed by the combined exercise training and trandolapril group. Maximum run time in the combined-treatment group was also significantly longer (17%) compared with the exercise-training-only group. Treatment with trandolapril only did not significantly affect \( \text{V} \text{O}_{2\text{peak}} \) or the maximum run time during the \( \text{V} \text{O}_{2\text{peak}} \) test.

OGTT responses. Table 3 details the fasting plasma levels of glucose, insulin, and free fatty acids in the experimental groups. Whereas the plasma glucose was lower in all three intervention groups than in the sedentary control, this was significant (13%, P < 0.05) only in the exercise-training-only group. Plasma insulin was significantly lower in the exercise-training-only and combined exercise training and trandolapril groups (both 34%) than in control. Compared with the sedentary control group, trandolapril treatment alone resulted in a significant 18% lowering of plasma free fatty acid levels, and exercise training, alone or in combination with trandolapril treatment, resulted in a 31% lowering of this variable.

The glucose and insulin responses during the OGTT in the experimental groups are shown in Fig. 1, with the calculated total areas under the curve (AUC) for these responses displayed in Fig. 2. In the trandolapril-treated and exercise-trained animals, glucose values...

Table 2. Peak oxygen consumption and maximum run time to fatigue after the 6-wk intervention periods

<table>
<thead>
<tr>
<th>Group</th>
<th>( \text{V} \text{O}_{2\text{peak}} ), ml·kg(^{-1})·min(^{-1} )</th>
<th>Maximum Run Time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obese sedentary</td>
<td>45.2 ± 1.8</td>
<td>8.8 ± 0.4</td>
</tr>
<tr>
<td>Obese trandolapril-treated</td>
<td>46.4 ± 0.5</td>
<td>9.9 ± 0.7</td>
</tr>
<tr>
<td>Obese exercise-trained</td>
<td>60.7 ± 2.2‡</td>
<td>11.4 ± 0.5*</td>
</tr>
<tr>
<td>Obese combined</td>
<td>59.2 ± 1.4‡</td>
<td>13.3 ± 0.8‡</td>
</tr>
</tbody>
</table>

Values are means ± SE for 5–9 animals/group. \( \text{V} \text{O}_{2\text{peak}} \), peak \( \text{O}_2 \) consumption. Maximum run time represents time to volitional fatigue during the assessment of \( \text{V} \text{O}_{2\text{peak}} \). *P < 0.05 vs. obese sedentary group, †P < 0.05 vs. obese trandolapril group, ‡P < 0.05 vs. all other groups.
were slightly lower than in control throughout the 60-min test period, but neither these values nor the glucose AUC values were significantly different from those in control. In the combined exercise training and trandolapril group, glucose values at all time points during the OGTT were 22–29% lower (P < 0.05) than in control animals, and the glucose AUC was significantly less (24%) than in control. Plasma insulin values in the trandolapril-only group were 25% lower (P < 0.05) than in control animals at 30 min, and the total insulin AUC was significantly less (20%) than the sedentary control value. Exercise training alone also resulted in a significant lowering of the insulin response during the OGTT, with 41 and 40% lower values at 30 and 60 min, respectively, and a 34% lower insulin AUC compared with that in control animals. A significant reduction in insulin responses during the OGTT was also realized in the obese animals that received the combination of exercise training and trandolapril treatment. Plasma insulin values at 15, 30, and 60 min were 26, 52, and 42% less, respectively, than in control animals, and the total insulin AUC was 40% less than in control.

The glucose-insulin index is defined as the product of the glucose and insulin AUCs, and a reduction in this value represents indirect evidence of improved in vivo peripheral insulin action (9). Trandolapril treatment alone induced a 30% lowering (P < 0.05) of the glucose-insulin index compared with that in sedentary control animals (Fig. 2). A significant diminution of this value (42%) was also realized with exercise training alone. However, the greatest reduction in the glucose-insulin index (55%), compared with that in sedentary control animals, was brought about by combined exercise training and trandolapril treatments, and this variable in the combined group was significantly lower than in both the trandolapril-treated group (35%) and exercise-trained group (22%).

Muscle glucose transport. To identify the cellular locus for these alterations in peripheral insulin action due to the interventions, we assessed insulin-mediated glucose transport activity, using 2-DG uptake, in isolated epitrochlearis and soleus muscles (Fig. 3). Rates of 2-DG uptake in the absence of insulin were not significantly different among the sedentary control, trandolapril-only, exercise-training-only, and combined exercise training and trandolapril groups in the epitrochlearis (108 ± 6, 115 ± 8, 106 ± 4, and 122 ± 19 pmol·mg muscle−1·20 min−1, respectively) or in the soleus (161 ± 16, 172 ± 18, 153 ± 7, and 190 ± 15 pmol·mg muscle−1·20 min−1, respectively). In the epitrochlearis, trandolapril treatment alone caused a significant increase (26%) in insulin-mediated 2-DG uptake (increase over basal), whereas exercise training alone also induced an increase (34%) in this parameter (Fig. 3A). The greatest enhancement in insulin-mediated 2-DG uptake was realized in the combined exercise training and trandolapril group, with a 67% increase compared with sedentary control. This variable was 33% greater (P < 0.05) in the combined group relative to the trandolapril-treated and exercise-trained groups.

A similar pattern was observed in the soleus muscle (Fig. 3B). Trandolapril treatment alone caused a significant 41% improvement in insulin action on glucose transport activity, and exercise training alone also resulted in a 39% increase in this variable in the soleus. As observed in the epitrochlearis, the greatest enhancement (107%) in insulin-mediated 2-DG uptake in the soleus was seen after combined exercise training and trandolapril treatments. This variable was 47 and 49% greater (P < 0.05), respectively, in the combined group

![Fig. 2. Effects of chronic treatment with trandolapril (Trand), exercise training (Exer), or trandolapril combined with exercise training (Combo) on incremental areas under curves (AUC) for glucose (A; mg·dl−1·min−1) and insulin (B; µU·ml−1·min−1) during an oral glucose tolerance test and glucose-insulin index (C; U × 10^6) in obese Zucker rats. Values are means ± SE for 5–9 animals/group. Sed, obese sedentary control group. Data for AUCs were taken from Fig. 1. Glucose-insulin index is calculated as product of glucose AUC and insulin AUC for each animal. ^a^P < 0.05 vs. Sed group. ^b^P < 0.05 vs. all other groups.](http://jap.physiology.org/Downloaded from 10.220.33.5 on October 1, 2017)
compared with the trandolapril-only and exercise-training-only groups.

GLUT-4 protein and enzyme responses. In the epitrochlearis, trandolapril treatment alone caused a significant increase (30%) in whole homogenate GLUT-4 protein level, exercise training alone induced a 48% increase, and the combination of exercise training and trandolapril treatment resulted in the greatest increase in GLUT-4 protein level (76%, \( P < 0.05 \) vs. all other groups) (Fig. 4A). Similarly, in the soleus muscle, trandolapril alone, exercise training alone, and the combination of these two interventions caused 30, 36, and 72% increases, respectively, in GLUT-4 protein (Fig. 4B). Finally, in the plantaris muscle, although GLUT-4 protein was not increased by trandolapril alone, this variable was significantly enhanced by 30 and 60%, respectively, in the exercise-training-only and the combined-intervention groups (Fig. 4C). These interventions did not induce significant alterations in GLUT-4 protein levels in the heart (data not shown).

Trandolapril treatment alone caused significant increases in both total hexokinase (24%) and citrate synthase activities (43%) in the epitrochlearis, but not in the soleus or plantaris (Table 4). Exercise training alone induced increased activities of hexokinase and citrate synthase in the epitrochlearis (25 and 30%, respectively) and plantaris (37 and 54%, respectively), whereas in the soleus this intervention significantly increased only citrate synthase activity (29%). Interestingly, the combination of exercise training and trandolapril treatment caused the greatest increases in hexokinase and citrate synthase in the soleus (36 and 42%,
factors associated with this syndrome, including hyper-
the development of the cardiovascular disease risk
resistance is thought to be involved in the etiology of
the use of combination therapy consisting of regular
nase activity.

increases in GLUT-4 protein expression and hexoki-
port in response to combined exercise training and ACE
inhibition were quantitatively associated with greater
improvements in whole body glucose tolerance and insulin-
action on skeletal muscle glucose transport activity.
Moreover, we have shown that these increases in
skeletal muscle GLUT-4 protein after exercise training or
chronic ACE inhibition, as chronic treatment of obese Zucker rats with bradyki-
inhibitor is unclear at the present time. Treatment with
ACE inhibitors, via inhibition of the kininase II reaction,
can reduce the degradation of the nonapeptide bradykinin and increase the circulating level of
this factor (34). It is now known that bradykinin administration can augment insulin-stimulated phos-
phorylation of skeletal muscle insulin receptors and
insulin receptor substrate-1, as well as the insulin-
stimulated association of insulin receptor substrate-1
and hexokinase activity after chronic ACE inhibition,
which is needed for insulin-mediated GLUT-4 translocation and
glucose transport (8). However, bradykinin is likely not
directly involved in the increased GLUT-4 protein level
and hexokinase activity after chronic ACE inhibition,
as chronic treatment of obese Zucker rats with bradyki-
inhibitor does not cause an increase in skeletal muscle
GLUT-4 or hexokinase (17). The reduction in angioten-
sin II, also a characteristic of ACE inhibition, may play
a role in this process, but this has not been tested
experimentally in the obese Zucker rat.

There was clearly additivity between the effects of
exercise training and chronic trandolapril treatment
for the induction of increases in GLUT-4 protein level
and insulin action on glucose transport activity in
skeletal muscle of the obese Zucker rats. This additivity
implies that these two interventions mediate their
effects on these variables via separate mechanisms. At
present, however, little information is available regarding
the mediators of the increased expression of skel-
etal muscle GLUT-4 protein after exercise training or
chronic ACE inhibition.

It is noteworthy that we were able to demonstrate
(Fig. 3) that exercise training by the obese Zucker rat
resulted in induction of GLUT-4 protein levels and in
enhanced insulin responsiveness of glucose transport
activity in both the epitrochlearis muscle, which consis-
t of predominantly type IIb fibers (28), and in the
soleus muscle, which consists of mainly type I fibers (1).
In previous investigations using a similar exercise
training protocol for the obese Zucker rat, enhanced in

Table 4. Total hexokinase and citrate synthase activities in skeletal muscles after the 6-wk intervention periods

<table>
<thead>
<tr>
<th></th>
<th>Epitrochlearis</th>
<th>Soleus</th>
<th>Plantaris</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total hexokinase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obese sedentary</td>
<td>5.1 ± 0.3</td>
<td>12.1 ± 0.4</td>
<td>8.2 ± 0.5</td>
</tr>
<tr>
<td>Obese trandolapril-treated</td>
<td>6.3 ± 0.2*</td>
<td>12.9 ± 0.1</td>
<td>8.2 ± 0.5</td>
</tr>
<tr>
<td>Obese exercise-trained</td>
<td>6.4 ± 0.4*</td>
<td>13.3 ± 1.1</td>
<td>11.2 ± 0.6†</td>
</tr>
<tr>
<td>Obese combined</td>
<td>6.9 ± 0.5*</td>
<td>16.4 ± 0.7†</td>
<td>14.0 ± 0.5‡</td>
</tr>
</tbody>
</table>

Citrate synthase
Obese sedentary | 67 ± 3        | 164 ± 4  | 155 ± 8  |
Obese trandolapril-treated | 96 ± 8* | 163 ± 13 | 178 ± 7  |
Obese exercise-trained | 87 ± 4* | 212 ± 9† | 238 ± 7‡ |
Obese combined | 93 ± 5* | 233 ± 7‡ | 270 ± 13‡ |

Values are means ± SE in nmol·mg protein⁻¹·min⁻¹ for 3–9 animals/group. *P < 0.05 vs. obese sedentary group; †P < 0.05 vs. obese trandolapril group. ‡P < 0.05 vs. all other groups.

In previous investigations demonstrating that, in the insulin-resistant, hyperinsulinemic, and dyslipidemic obese Zucker rat, exercise training by treadmill running (2, 3, 6, 9, 13, 19, 37) or chronic administration of the ACE inhibitor trandolapril (20) results in significant improvements in whole body insulin action on peripheral disposal of a glucose load and in insulin action on skeletal muscle glucose transport activity. Moreover, we have shown that these increases in insulin action on skeletal muscle glucose transport were associated with upregulation of the GLUT-4 glucose transporter isoform (2, 6, 13, 20) and in total hexokinase (19, 20, 32). More importantly, we have demonstrated for the first time that greater improvements in whole body glucose tolerance and insulin-stimulated muscle glucose transport activity in the obese Zucker rat could be achieved through the combination of exercise training and trandolapril treatment than with either intervention individually, and that these greater improvements in muscle glucose transport in response to combined exercise training and ACE inhibition were quantitatively associated with greater increases in GLUT-4 protein expression and hexokinase activity.

These findings could have important implications for the use of combination therapy consisting of regular aerobic exercise and concomitant ACE inhibition in treatment of the insulin resistance syndrome. Insulin resistance is thought to be involved in the etiology of the development of the cardiovascular disease risk factors associated with this syndrome, including hyper-
tension and dyslipidemia (11). Our observation that greater improvements in whole body glucose tolerance and skeletal muscle insulin action could be realized with this combination therapy in the obese Zucker rat compared with either intervention individually indicates that similar reductions in cardiovascular disease risk could also be brought about with this combined-intervention approach. It is clear that the present results support future clinical investigations involving combined aerobic exercise training and ACE inhibition in human populations displaying characteristics of insulin resistance syndrome, especially essential hypertension, glucose intolerance, and insulin resistance.

Our previous finding that chronic trandolapril treatment of obese Zucker rats induces increases in GLUT-4 protein, total hexokinase activity, and insulin action on glucose transport in the epitrochlearis muscle (20) has been confirmed in the present study and can now be extended to the soleus muscle (Fig. 4). The underlying cause for this induction of these factors involved in glucose transport and phosphorylation by an ACE inhibitor is unclear at the present time. Treatment with ACE inhibitors, via inhibition of the kininase II reaction (12), can reduce the degradation of the nonapeptide bradykinin and increase the circulating level of this factor (34). It is now known that bradykinin administration can augment insulin-stimulated phosphorylation of skeletal muscle insulin receptors and insulin receptor substrate-1, as well as the insulin-stimulated association of insulin receptor substrate-1 and phosphatidylinositol-3-kinase (7), all of which are needed for insulin-mediated GLUT-4 translocation and glucose transport (8). However, bradykinin is likely not directly involved in the increased GLUT-4 protein level and hexokinase activity after chronic ACE inhibition, as chronic treatment of obese Zucker rats with bradyki-
inhibitor itself does not cause an increase in skeletal muscle GLUT-4 or hexokinase (17). The reduction in angioten-
sin II, also a characteristic of ACE inhibition, may play
a role in this process, but this has not been tested
experimentally in the obese Zucker rat.

respectively) and plantaris (71 and 74%, respectively)
(both P < 0.05 vs. all other groups), whereas in the
epitrochlearis the increases in these variables in the combination group (35 and 39%, respectively) were not significantly greater than those observed in the individ-
ual intervention groups. These variables were not significantly altered in the hearts of obese animals in
the three intervention groups compared with those in
sedentary control animals (data not shown).

DISCUSSION

In the present study, we have confirmed the findings of previous investigations demonstrating that, in the insulin-resistant, hyperinsulinemic, and dyslipidemic obese Zucker rat, exercise training by treadmill running (2, 3, 6, 9, 13, 19, 37) or chronic administration of the ACE inhibitor trandolapril (20) results in significant improvements in whole body insulin action on peripheral disposal of a glucose load and in insulin action on skeletal muscle glucose transport activity. Moreover, we have shown that these increases in insulin action on skeletal muscle glucose transport were associated with upregulation of the GLUT-4 glucose transporter isoform (2, 6, 13, 20) and in total hexokinase (19, 20, 32). More importantly, we have demonstrated for the first time that greater improvements in whole body glucose tolerance and insulin-stimulated muscle glucose transport activity in the obese Zucker rat could be achieved through the combination of exercise training and trandolapril treatment than with either intervention individually, and that these greater improvements in muscle glucose transport in response to combined exercise training and ACE inhibition were quantitatively associated with greater increases in GLUT-4 protein expression and hexokinase activity.

These findings could have important implications for the use of combination therapy consisting of regular aerobic exercise and concomitant ACE inhibition in treatment of the insulin resistance syndrome. Insulin resistance is thought to be involved in the etiology of the development of the cardiovascular disease risk factors associated with this syndrome, including hyper-
tension and dyslipidemia (11). Our observation that greater improvements in whole body glucose tolerance and skeletal muscle insulin action could be realized with this combination therapy in the obese Zucker rat compared with either intervention individually indicates that similar reductions in cardiovascular disease risk could also be brought about with this combined-intervention approach. It is clear that the present results support future clinical investigations involving combined aerobic exercise training and ACE inhibition in human populations displaying characteristics of insulin resistance syndrome, especially essential hypertension, glucose intolerance, and insulin resistance.

Our previous finding that chronic trandolapril treatment of obese Zucker rats induces increases in GLUT-4 protein, total hexokinase activity, and insulin action on glucose transport in the epitrochlearis muscle (20) has been confirmed in the present study and can now be extended to the soleus muscle (Fig. 4). The underlying cause for this induction of these factors involved in glucose transport and phosphorylation by an ACE inhibitor is unclear at the present time. Treatment with ACE inhibitors, via inhibition of the kininase II reaction (12), can reduce the degradation of the nonapeptide bradykinin and increase the circulating level of this factor (34). It is now known that bradykinin administration can augment insulin-stimulated phosphorylation of skeletal muscle insulin receptors and insulin receptor substrate-1, as well as the insulin-stimulated association of insulin receptor substrate-1 and phosphatidylinositol-3-kinase (7), all of which are needed for insulin-mediated GLUT-4 translocation and glucose transport (8). However, bradykinin is likely not directly involved in the increased GLUT-4 protein level and hexokinase activity after chronic ACE inhibition, as chronic treatment of obese Zucker rats with bradyki-
inhibitor itself does not cause an increase in skeletal muscle GLUT-4 or hexokinase (17). The reduction in angioten-
sin II, also a characteristic of ACE inhibition, may play
a role in this process, but this has not been tested
experimentally in the obese Zucker rat.

There was clearly additivity between the effects of exercise training and chronic trandolapril treatment for the induction of increases in GLUT-4 protein level and insulin action on glucose transport activity in skeletal muscle of the obese Zucker rats. This additivity implies that these two interventions mediate their effects on these variables via separate mechanisms. At present, however, little information is available regarding the mediators of the increased expression of skel-
etal muscle GLUT-4 protein after exercise training or
chronic ACE inhibition.

It is noteworthy that we were able to demonstrate
(Fig. 3) that exercise training by the obese Zucker rat
resulted in induction of GLUT-4 protein levels and in
enhanced insulin responsiveness of glucose transport
activity in both the epitrochlearis muscle, which consis-
t of predominantly type IIb fibers (28), and in the
soleus muscle, which consists of mainly type I fibers (1).
In previous investigations using a similar exercise
training protocol for the obese Zucker rat, enhanced in

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vitro insulin responsiveness of glucose transport activity was observed only in the epitrochlearis and not in the soleus (13), despite the fact that GLUT-4 protein expression was significantly increased in both muscles at this time point (2, 6, 13). One major difference between the present study and previous investigations is that, in these earlier studies, glucose transport was assessed at least 48 h after the final bout of exercise, whereas in our study this variable was determined only 24 h postexercise. It is possible that some factor developed between the 24- and 48-h postexercise time points that prevented the enlarged GLUT-4 pool in the exercise-trained soleus from translocating to the sarcolemma (13) and allowing a greater rate of insulin-stimulated glucose transport. Alternatively, this training-induced increase in insulin action in the obese Zucker rat may have disappeared between 24 and 48 h postexercise. Further investigations are needed to determine what underlies these observations.

Although the maximum run times during the VO2peak tests were significantly longer in both exercise-trained obese groups than in sedentary control animals, this time was nearly 2 min (17%) longer in the exercise-trained obese animals that received trandolapril compared with those obese animals that underwent exercise training alone. This difference in performance could not be attributable to differences in peak aerobic capacity or cardiac mass (a crude index of cardiac output capacity), as these variables were essentially the same in both groups. A more viable explanation relates to the finding that GLUT-4 protein was greatest in epitrochlearis, soleus, and plantaris muscles and total hexokinase and citrate synthase activities were greatest in the soleus and plantaris muscles of the combined exercise training and trandolapril group. The increases in these variables would likely enhance oxidative substrate utilization by these locomotor muscles during the treadmill test and thereby increase endurance.

There is increasing evidence that elevated circulating free fatty acid levels may be involved in the multifactorial pathogenesis of insulin-resistant states (5). Trandolapril alone and exercise training alone both led to reductions in plasma free fatty acids that were accompanied by an enhancement of insulin-stimulated skeletal muscle glucose transport activity, consistent with the hypothesis that the diminution of free fatty acids may contribute, at least in part, to the improvements in insulin action after these respective interventions. However, the combination of exercise training and trandolapril treatment did not further reduce plasma free fatty acid concentrations. This indicates that the additional improvement in insulin action on skeletal muscle glucose transport activity observed in this combined group relative to the exercise-training-only group cannot be ascribed to effects mediated by free fatty acids and must be due to other factors, such as the further enhancement of muscle GLUT-4 protein levels.

In conclusion, we have shown that exercise training alone and chronic administration of the ACE inhibitor trandolapril alone both lead to increased whole body and skeletal muscle insulin action in the severely insulin-resistant, hyperinsulinemic, and dyslipidemic obese Zucker rat. However, the greatest improvements in whole body glucose tolerance and skeletal muscle insulin action in this animal model were observed when exercise training and ACE inhibition were performed in combination. Moreover, these effects on skeletal muscle insulin action were associated with increases in the muscle level of GLUT-4 protein and total hexokinase activity. These results indicate that this combination of therapeutic interventions may represent a more effective way of reducing cardiovascular disease risk factors in this animal model than either intervention individually.

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Address for reprint requests and other correspondence: E. J. Henriksen, Dept. of Physiology, Ina E. Gittings Bldg. #93, Univ. of Arizona, Tucson, AZ 85721-0093 (E-mail: ejhenrik@u.arizona.edu).

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