Exercise-induced increase in skeletal muscle vasodilatory responses in obese Zucker rats

Lusha Xiang, Jay Naik, and Robert L. Hester

Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, Mississippi

Submitted 13 October 2004; accepted in final form 8 December 2004

Xiang, Lusha, Jay Naik, and Robert L. Hester. Exercise-induced increase in skeletal muscle vasodilatory responses in obese Zucker rats. Am J Physiol Regul Integr Comp Physiol 288: R987–R991, 2005. First published December 16, 2004; doi:10.1152/ajpregu.00702.2004.—The purpose of this study was to test the hypothesis that exercise training improves microvascular function in obese Zucker rats, a model of obesity and type II diabetes. Animals were divided into four age-matched groups: lean sedentary (LS), lean exercise (LE), obese sedentary (OS), and obese exercise (OE). The exercise groups were treadmill-exercised from 5 to 11 wk of age, including a 2-wk acclimation period. Mean arterial pressure (MAP) was not significantly different between any of the groups. The OS had significantly higher mean body weight, blood glucose, insulin, IL-6, and leptin levels compared with the LS, whereas the OE had significantly lower blood glucose, insulin, and IL-6 levels compared with the OS. Functional hyperemia and endothelial-dependent vasodilation were tested in the spinotrapezius muscle using intravital microscopy. Functional hyperemia and acetylcholine (0.1 μM, 1 μM, and 10 μM) responses were significantly attenuated in OS compared with the LS, while the contraction and ACh-induced (1 μM and 10 μM) vasodilation were significantly increased in both LE and OE compared with the sedentary animals. These results suggest that exercise training can improve vascular function in this model of type II diabetes. Moreover, the impaired vasodilation observed in 11-wk-old OZR suggests that the microvascular dysfunction is not likely due to an elevated blood pressure.

Functional hyperemia; arteriole; diabetes

TYPE II DIABETES MELLITUS is often associated with obesity, insulin resistance, hyperglycemia, and other cardiovascular diseases. Collectively, these endocrine and metabolic disturbances are described as “metabolic syndrome”. Much of the morbidity and mortality associated with diabetes is attributable to macro- and microvascular complications. In addition, endothelial dysfunction is a component in the development of diabetic vascular disease. However, the pathophysiological processes linking obesity and type II diabetes and microvascular dysfunction are unclear.

Exercise training has been advocated as a nonpharmacological measure for the treatment of obesity and type II diabetes. Numerous studies have reported that regular aerobic exercise training not only lowers mean blood pressure (4, 6) but also improves insulin sensitivity and glucose homeostasis in diabetic rodents and humans (19, 32). At the microcirculatory level, exercise training has also been shown to enhance adrenergic (24) and flow-dependent dilation (22) and decrease peripheral vascular resistance (7) in skeletal muscle arterioles. To study the effects of chronic exercise on obesity and diabetes, Arvola et al. (2) exercised obese Zucker rats (OZRs) for 22 wk and found an enhanced arterial vasorelaxation, lower blood pressure, and decreased blood glucose (2). However, it is unclear as to whether the enhanced vasorelaxation was secondary to an exercise-training-mediated decrease in blood pressure. No in vivo data on microvascular function in skeletal muscle after exercise training have been reported. Indeed, the effects of exercise training on the microcirculation in skeletal muscle in a model of metabolic syndrome are unknown.

Owing to mutations in the leptin receptor gene, the OZR is characterized by greater body fat, insulin resistance, and a gradual development of moderate hypertension (17, 23). Several recent reports have demonstrated impaired skeletal muscle microvascular function in OZRs (15, 16). Because exercise has been reported to improve vascular function, we tested the hypothesis that exercise training will improve functional hyperemia and agonist-induced, endothelial-dependent vasodilation in OZRs. To control for the effects of blood pressure, we studied 11- to 12-wk-old obese and the lean Zucker rats after a 4-to 5-wk training period. Mean arterial pressure (MAP), blood hormones (glucose, insulin, IL-6, and leptin), and arteriolar dilation to the endothelial-dependent stimuli, muscle contraction and ACh were examined.

METHODS

Animals. The experimental protocols for this study were approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center and were carried out in accordance with the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health and the guidelines of the Animal Welfare Act. All the rats were housed 2 or 3 animals per cage at 22°C (12:12-h light-dark cycle) with free access to regular food and water.

Exercise training protocol. Five-week-old obese and lean Zucker rats were acquired from Harlan Laboratories (Jerusalem, Israel; n = 66). The animals were divided into four groups, lean sedentary (LS) and lean exercise (LE), obese sedentary (OS), and obese exercise (OE). The exercising animals were subjected to a treadmill exercise protocol consisting of 4–5 wk of exercise at 24 m/min with a 10-day acclimation period. The rats were exercised in a temperature (22°C) and humidity (55%)-controlled room using a Columbus Instruments (Columbus, OH) treadmill.

Blood glucose, insulin, IL-6, and leptin. A set of the lean and obese Zucker rats (n = 26) was used to measure blood hormones (glucose, insulin, IL-6, and leptin) after the same exercise protocol. After 4 h of food restriction, rats from all four groups were decapiated for the collection of blood samples. Blood samples from rats in the exercise groups were taken at least 24 h after their last exercise period. Insulin and leptin were analyzed by radioimmunoassay kits (Linco Re-

Address for reprint requests and other correspondence: R. Hester, Dept. of Physiology and Biophysics, Univ. of Mississippi Medical Center, 2500 North State St., Jackson, MI 39216–4505 (E-mail: rheser@physiology.umsmed.edu).

http://www.ajpregu.org 0363-6119/05 $8.00 Copyright © 2005 the American Physiological Society
search, St. Louis, MO). Glucose was analyzed on a Beckman clinical chemistry analyzer (Beckman Instruments, Fullerton, CA). The concentration of IL-6 was determined by the ELISA method (Quantikine kit, R&D Systems, Minneapolis, MN). The exercise training decreased body weight significantly in both groups; n = 15 for LS, n = 16 for LE, n = 15 for obese sedentary (OS), and n = 14 for obese exercise (OE).

Microcirculatory surgical preparation. Animals were prepared for microcirculatory experiments 24 h after measurement of blood pressure. The muscles were prepared for experimental observation as previously described (3, 26). In brief, rats were anesthetized with pentobarbital sodium (65 mg/kg ip), and the trachea was intubated. Animals spontaneously breathed a gas mixture containing 30% oxygen and 70% nitrogen. At all times during the surgery and subsequent microcirculatory experiments 24 h after measurement of blood pressure, arterial pressure was continuously monitored for 2 h, and the arterial pressure over the last hour was averaged.

Measurement of blood pressure. Rats were anesthetized with 2% isoflurane and surgically instrumented with arterial catheters (PE-50 tubing) in the left carotid artery for blood pressure monitoring. All catheters were tunneled to the back of the neck and exteriorized. Blood pressure measurements were made 24 h after the placement of the catheter. To minimize the residual effect of an acute bout of exercise, arterial pressure was measured in conscious rats the following day with a pressure transducer connected to a computerized data collection system. The resolution of this system was 0.30. The microscopic image was televised with a Dage closed-circuit television camera and displayed on a Sony monitor. The magnification of the image was ×1,000 from the tissue to the monitor screen. Vessel diameter was measured by using a Texas A&M (College Station, TX) video analyzer modified to function as a video micrometer. With the use of this device, we positioned two movable lines on the inside walls of the vessel, and a DC voltage proportional to the line separation was recorded using a computerized data-collecting system. The resolution of this system was ±1 μm.

Experimental protocol. Animals were allowed to stabilize for 15–30 min after completion of the surgical procedure. A segment of the arteriolar vascular arcade was selected for analysis. Two hooked silver-silver chloride electrodes (Harvard Instruments) were placed at each end of the spinotrapezius and were connected to a Grass S44 stimulator. Diameters of each of the vessels were obtained in the resting muscle and immediately after 2 min of electrical stimulations (4–5V, 1 Hz). After the vessel had returned to its resting diameter, vasodilatory responses to serial concentrations of acetylcholine (ACh; 0.1 μM, 1 μM, and 10 μM) were determined. Arteries were washed and allowed to return to baseline diameter between ACh concentrations.

Analytic and statistical methods. Arterial diameter data were collected at 1 Hz using a computer equipped with a Metrabyte Dash 8-bit analog-to-digital converter and stored to disk for later analysis. Statistical significance of data was assessed using either paired t-test or two-way ANOVA. Significant main effects were analyzed using the Student-Newman-Keuls post hoc test. All data are means ± SD. Statistical significance was accepted at P < 0.05.

RESULTS

Physical characteristics. The obese animals had significantly higher body weights than the lean controls. After the exercise training protocol, body weight was significantly lower in the exercised animals compared with the sedentary animals. The LS animals were significantly heavier than the LE animals (P < 0.01) and the OS animals were significantly heavier than the OE animals (P < 0.02) (Fig. 1). MAP was not significantly different between any of the groups (Fig. 2). There were no differences in heart rate between any of the groups (data not shown).

Blood hormones. Fasting blood glucose, insulin, IL-6, and leptin levels are presented in Table 1. Obese sedentary animals

![Graph showing Body Weight gains over 7 weeks](image1)

**Fig. 1.** There was a progressive increase in body weight over the 7-wk period. The body weight of the lean exercise (LE) animals was significantly lower than the lean sedentary (LS) at the end of the 7-wk period. *The weights of the obese animals were significantly higher than the lean animals. †The exercise training decreased body weight significantly in both groups; n = 15 for LS, n = 16 for LE, n = 15 for obese sedentary (OS), and n = 14 for obese exercise (OE).

![Graph showing MAP changes](image2)

**Fig. 2.** There were no significant differences in mean arterial pressure (MAP) between any of the groups; n = 8 for LS, n = 7 for LE, n = 7 for OS, and n = 5 for OE.

Table 1. Blood hormone levels in LS, LE, OS, and OE animals

<table>
<thead>
<tr>
<th></th>
<th>LS</th>
<th>LE</th>
<th>OS</th>
<th>OE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dl</td>
<td>151 (SD 7)</td>
<td>150 (SD 13)</td>
<td>217 (SD 56)*</td>
<td>156 (SD 12)†</td>
</tr>
<tr>
<td>Insulin, μU/ml</td>
<td>81 (SD 30)</td>
<td>44 (SD 15)</td>
<td>604 (SD 210)*</td>
<td>392 (SD 134)#</td>
</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>53 (SD 11)</td>
<td>35 (SD 8)†</td>
<td>79 (SD 30)</td>
<td>34 (SD 12)†</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>7 (SD 2)</td>
<td>3 (SD 1)</td>
<td>42 (SD 4)*</td>
<td>44 (SD 6)*</td>
</tr>
</tbody>
</table>

*Significant difference between lean and obese animals; †significant difference between sedentary and exercised animals; #significant difference between sedentary and exercised obese animals. LS, lean sedentary; LE, lean exercise; OS, obese sedentary; OE, obese exercise; n = 6 for each group.
had higher blood glucose levels compared with lean rats \((P = 0.0008)\). Exercise training resulted in a decrease in blood glucose \((P = 0.003)\) in obese animals, but there was no change in lean rats. Blood insulin was significantly elevated in obese rats compared with lean animals \((P < 0.001)\). Exercise training decreased blood insulin compared with sedentary rats \((P = 0.025)\). There was no difference in IL-6 levels between the lean and obese animals \((P = 0.10)\). However, IL-6 levels in the sedentary animals were significantly higher than exercised animals \((P < 0.001)\). Leptin levels were greater in obese rats than in lean rats \((P < 0.001)\). Exercise training had no effect on serum leptin levels \((P = 0.6)\).

**Functional vasodilation.** Basal arteriolar diameters were 15 \((1 \text{ SD} \mu \text{m (} n = 8)\); 14 \((1 \text{ SD} \mu \text{m (} n = 10)\); 25 \((2 \text{ SD} \mu \text{m (} n = 8)\); and 19 \pm (3 \text{ SD} \mu \text{m (} n = 6)\) for LS, LE, OS, and OE rats, respectively. Resting arteriolar diameter was significantly greater in obese compared with lean rats \((P < 0.001)\). Exercise training decreased resting arteriolar diameter in obese rats \((P = 0.02)\). However, exercise training had no effect on resting arteriolar diameter in lean animals. Figure 3 presents the vasodilatory response to muscle contraction in arcade arterioles of the spinotrapezius muscle. In all cases, muscle contraction resulted in a significant increase in luminal diameter. The functional hyperemic response was significantly blunted in obese animals compared with their lean controls \((P < 0.001)\). Moreover, exercise training enhanced the vasodilatory response to muscle contraction in both lean and obese animals \((P < 0.001)\). The arteriolar vasodilatory responses to topical administration of acetylcholine are presented in Fig. 4. The administration of ACh resulted in a concentration-dependent increase in luminal diameter in animals from both groups. Vasodilation in response to ACh was significantly impaired in obese animals at all concentrations compared with lean controls. *Exercise training augmented the vasodilatory response to ACh at higher concentrations compared with sedentary animals.

**DISCUSSION**

Obesity is associated with hypertension, dyslipidemia, elevated blood glucose, reduced insulin sensitivity, and development of cardiovascular diseases—a collection of conditions often referred to as metabolic syndrome. The OZR has been studied as a model of obesity and type II diabetes. As shown in Table 1, the OZR has elevated fasting blood glucose, insulin, and IL-6 levels, consistent with what is observed in humans suffering from type II diabetes. Similar results have shown that 13- to 15-wk OZRs have fasting hyperglycemia and hyperinsulinemia relative to LZR \((14, 30)\). As shown in Figs. 3 and 4, both functional hyperemia and ACh-induced endothelial-dependent vasodilation are impaired in OS Zucker rats, suggesting impaired endothelial function. Numerous studies have shown an attenuated endothelium-dependent dilation in diabetic patients and animals \((10, 20)\), but the mechanisms responsible for the endothelial dysfunction are unclear.

In the present study, we used the spinotrapezius microcirculation because no other postural muscle is suitable. Previous findings suggested that treadmill running does not recruit the spinotrapezius muscle; thus, the training-induced arteriolar adaptations produced in this muscle may be unrelated to the augmented local blood flow or muscle metabolism \((28)\). However, Lash and Bohlen \((26)\) demonstrated that 8–10 wk of treadmill exercise greatly increased the functional dilation in the Sprague-Dawley rat spinotrapezius muscle in response to 1–8 Hz of electrical stimulation \((25)\). Consistent with this finding, we observed during this study an increase in functional hyperemia in the LE animals. Levels of glucose, insulin, and IL-6, and blood pressure of LE animals were similar to the LS animals, and these results suggest that the enhanced functional hyperemic response was a training effect; that is, the muscles did exercise. This observation suggests that the improvement in the functional hyperemic responses in lean animals was not due to glucose, insulin, or blood pressure changes. Indeed, Delp and Laughlin \((11)\) showed that endothelium-dependent, ACh-mediated dilation of the rat aorta was enhanced by 4 wk of treadmill training. Whether exercise training-mediated improvements in endothelial function in obese animals occurs through direct muscle activity, an alteration in the hormonal milieu or both is unknown. Future studies will examine vascular function in the cremaster muscle (nonexercise muscle) in both lean and obese trained rats to test the global effects of exercise.

Several studies support a role for hyperglycemia in endothelial dysfunction. Jin and Bohlen \((21)\) showed that acute
hyperglycemia results in inhibition of acetylcholine and flow-mediated vasodilation in the mesenteric microcirculation. In addition, direct exposure of human aortic endothelial cells to high glucose levels results in nitration of the PGI synthase enzyme and an inhibition of prostacyclin release (8, 34). Finally, PG2 and PGE2 release was reported to be diminished in a fructose feeding model of elevated glucose levels (29). From these studies, we would hypothesize that endothelial dysfunction in OZR might, at least partly, result from the increased blood glucose and decreased insulin sensitivity present in these animals. Further studies are needed to probe the mechanisms of hyperglycemia-induced endothelial dysfunction.

A well-established response to exercise training in type II diabetes is an improved glucose tolerance and enhanced skeletal muscle insulin sensitivity (19, 33). As mentioned before, decreased glucose or improved insulin sensitivity results in improved endothelial function. In the current study, we hypothesize that, in addition to the tissue-specific effects of exercise, the normalization of blood hormones in OE (Table 1) might have global effect to improve vascular function. In fact, Arvola et al. (2) showed that long-term exercise in OZR enhanced the ACh-induced relaxation of mesenteric and carotid artery rings (2). The changes in vascular reactivity in vessels occurred in the presence of reduced blood glucose, cholesterol, and triglyceride levels, suggesting a global effect of the exercise on vascular reactivity. Moreover, Green et al. (18) found that lower limb exercise caused an increase in the production of NO in the resting forearm. Thus changes in circulating factors may be responsible for the improvement in vascular function in response to exercise.

IL-6 production and subsequent release by skeletal muscle plays a role in the regulation of glucose homeostasis in insulin-sensitive tissue. IL-6 is upregulated in insulin-resistant tissue in an attempt to overcome such a metabolic dysfunction (13). Numerous studies have shown that impaired glucose tolerance is tightly associated with increased serum concentrations of IL-6 (9, 12). Although the mechanism of the increase of IL-6 in diabetes is uncertain, it has been postulated that the increased IL-6 provides a potential link between type II diabetes and impaired endothelial function. Treatments for type II diabetes that decrease IL-6 levels, such as weight reduction, have been associated with improved endothelial function (1). We hypothesize that the decreased IL-6 levels associated with improved hyperglycemia and insulin resistance after exercise training may partly account for the enhanced endothelial function.

In addition, blood leptin is tightly associated with fat mass, and exercise-induced weight loss leads to a decrease in leptin levels (27). In the present study, the leptin levels in OZR were significantly higher than lean Zucker rats and remained unchanged after exercise (Table 1). These results are consistent with a study in type II diabetic patients that demonstrated that 8 wk of intensive training did not change the blood leptin level, although a marked reduction in abdominal fat and an increase in insulin sensitivity were observed (5). These results show that the improved endothelial function in OE is unrelated to the blood leptin level.

In the current studies the arterioles in the OS animals had a significantly larger basal diameter. These changes could be due to a physiological response such as diminished vascular tone, surgical procedures, or selection criteria. In our experiments, we believe that our selection criterion was responsible for the larger basal diameters. All of the arterioles that we studied were in the same order of arcade vessels. Usually, the smaller segments were chosen for study because the vascular walls can be seen clearly. However, in some OS animals, the smaller segments cannot be observed clearly because of the thick connective tissue around them. However, these findings should not bias the results, as the OS animals had a significantly smaller absolute and relative vasodilatory response compared with all other groups.

Several studies showed that obese rats are mildly hypertensive at 20 wk of age compared with their lean controls (31) and have hypertension by the age of 22 wk (2). The OZR animals in our study had not yet developed the elevated blood pressure seen in older animals. In addition, exercise training had no effect on resting blood pressure in either group (Fig. 2). The differences in the functional hyperemia and ACh response between OZRs and LZRs, without significant differences in blood pressure, suggest that the impaired endothelial function in obese rats is not secondary to elevations in blood pressure. To our knowledge, this is the first study to provide evidence that the microvascular dysfunction observed in the OZR is not likely due to an elevated blood pressure. Future studies using 24-h continuous pressure measurements are needed to verify that 11- to 12-wk-old OZRs are not hypertensive and to determine whether there are differential blood pressure responses between OZRs and LZRs during the exercise period.

Summary and conclusions. The present study demonstrated an impaired arteriolar dilation to both ACh and muscle contraction in the spinotrapezius muscle in a model of obesity and diabetes. Exercise training augmented the endothelium-dependent vasodilation in animals from both groups. Moreover, these studies have shown that obese rats exhibit increased blood glucose, insulin, and IL-6 levels, and that 4 wk of exercise training lowered insulin and glucose levels in obese rats, while decreasing IL-6 levels in animals from both groups. Additional experiments are needed to determine the contribution of the cyclooxygenase and nitric oxide synthase pathways to the enhanced endothelium-mediated relaxation and whether improved glucose and insulin homeostasis in OZR is responsible for the improved vascular function.

ACKNOWLEDGMENTS

The authors thank Carmen Adcock, Jennifer Dearman, Jennifer Harris, and Radu Iiescui for technical help in these experiments.

GRANTS

These studies were supported by National Institutes of Health Grants HL-51971 and HL-63958.

REFERENCES


