Functional adaptations in the skeletal muscle microvasculature to endurance and interval sprint training in the type 2 diabetic OLETF rat

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1Department of Biomedical Sciences, University of Missouri, Columbia, Missouri; 2Department of Nutrition and Exercise Physiology, University of Missouri, Columbia, Missouri; 3Department of Internal Medicine, University of Missouri, Columbia, Missouri; 4Department of Medical Pharmacology and Physiology, University of Missouri, Columbia, Missouri; 5Dalton Cardiovascular Research Center, University of Missouri, Columbia, Missouri; and 6Harry S. Truman Veterans Affairs Memorial Hospital, Columbia, Missouri

Submitted 5 July 2012; accepted in final form 21 August 2012

Martin JS, Padilla J, Jenkins NT, Crissey JM, Bender SB, Rector RS, Thyfault JP, Laughlin MH. Functional adaptations in the skeletal muscle microvasculature to endurance and interval sprint training in the type 2 diabetic OLETF rat. J Appl Physiol 113: 1223–1232, 2012. First published August 23, 2012; doi:10.1152/japplphysiol.00823.2012.—Prevention and treatment of type 2 diabetes includes recommendation to perform aerobic exercise, but evidence indicates that high-intensity exercise training may confer greater benefit. Unique motor recruitment patterns during exercise elicit spatially focused increases in blood flow and subsequent adaptations. Therefore, using 20-wk-old Otsuka Long Evans Tokushima fatty (OLETF) rats with advanced insulin resistance, we examined whether 12 wk of exercise protocols that elicit different motor unit recruitment patterns, endurance exercise (EndEx), and interval sprint training (IST) induce spatially differential effects on endothelial-dependent dilation to acetylcholine (ACh; 1 nM–100 μM) and vasoreactivity to insulin (1–1,000 μIU/ml) in isolated, pressurized skeletal muscle resistance arterioles. Compared with sedentary OLETF rats, EndEx enhanced sensitivity to ACh in second-order arterioles perfusing the “red” (G2A-R) and “white” (G2A-W) portions of the gastrocnemius (EC50: +36.0 and +31.7%, respectively), whereas IST only increased sensitivity to ACh in the G2A-R (+35.5%). Significant heterogeneity in the vasomotor response to insulin was observed between EndEx and IST as mean clearance during a hyperglycemic challenge (10, 15, 18), insulin signaling in skeletal muscle has been a major focus of the adaption to interventions aimed at improving insulin sensitivity. However, nutritive blood flow to metabolically active tissue is an important component of insulin-mediated glucose uptake in skeletal muscle. Indeed, insulin-mediated increases in skeletal muscle blood flow contribute to as much as 40% of total insulin-stimulated glucose uptake (5, 6). Importantly, vascular reactivity to insulin, and the resulting increase in skeletal muscle perfusion, is impaired in obesity and type 2 diabetes mellitus (T2D), ultimately attenuating nutritive blood flow and contributing to insulin resistance (20, 22, 40).

The Otsuka Long-Evans Tokushima fatty (OLETF) rat is a model of T2D, characterized by a mutated cholestykinin-1 receptor that results in impaired within-meal satiety and hyperphagia (19). OLETF rats are known to spontaneously develop obesity and insulin resistance by 20 wk of age and continue to progress to overt T2D over time (9, 19, 29). Our laboratory has previously shown that access to voluntary running wheels beginning at 4 wk of age can effectively prevent the development of insulin resistance, suggesting that a lack of physical activity is a significant contributor to the development of T2D in this model (31). However, the ability of exercise, in line with what may be prescribed for T2D treatment in humans, to improve microvascular insulin sensitivity in the OLETF rat has not been determined.

The current standard of care for persons with insulin resistance and T2D includes recommendation for lifestyle modification (2). Among these lifestyle changes, the American Diabetes Association recommends 150 min/wk of moderate physical activity (i.e., aerobic exercise) for the prevention and treatment of T2D (2). However, there is evidence of greater benefit from high-intensity exercise [e.g., interval sprint training (IST)] that recruits both type I (slow-twitch) and II (fast-twitch) motor units (8, 38). Skeletal muscle fibers can be grouped into three general phenotypes based on contractile and metabolic properties: slow-twitch oxidative (“red”), fast-twitch oxidative, glycolytic (“red”), and fast-twitch glycolytic (“white”) fibers (4). Importantly, some intrinsic vascular functions of skeletal muscle arterioles differ among arterioles perfusing each fiber type (1, 30, 32). Moreover, these fiber-type differences interact with skeletal muscle fiber recruitment patterns during exercise, eliciting spatially focused increases in blood flow and subsequent adaptive changes (12, 28). Therefore, similar complexity is likely involved in the adaptations to glucose...
exercise for T2D treatment that utilize different muscle-fiber recruitment patterns (i.e., sprint vs. aerobic training).

Endurance exercise (EndEx) and IST have both been shown to alter the function of arteries perfusing skeletal muscle (21, 23, 27, 28). These exercise-induced adaptations appear to be concentrated in the muscle tissue having the greatest relative increase in activity during training sessions (3, 12, 17, 37). A major player in the adaptive response to exercise in the skeletal muscle arterioles is blood flow shear stress (26). Previous investigations by Laughlin and Armstrong (24) have demonstrated that blood flow to the fast-twitch red muscle tissue of the gastrocnemius is increased significantly from preexercise values with both low- (e.g., EndEx) and high-intensity (e.g., IST) exercise. However, only high-intensity exercise appears to significantly increase blood flow to the fast-twitch, white muscle tissue of the gastrocnemius (24). Therefore, exercise training protocols that involve different motor unit recruitment patterns (EndEx vs. IST) when used in the treatment of advanced insulin resistance may have differential effects on endothelial-dependent vasoreactivity to acetylcholine (ACh) and insulin within and among the skeletal muscle arteries and arterioles of the OLETF rat hindlimb. We hypothesized that EndEx would have beneficial effects on primary and secondary arteriolar vasoreactivity and vessel function in the arteries perfusing “red” gastrocnemius muscle fibers, whereas IST would improve the function of arteries perfusing both “red” and “white” regions of the gastrocnemius. The rationale for this hypothesis is that IST exercise bouts were expected to produce larger relative increases in activity of the white fibers and to recruit a larger proportion of the gastrocnemius muscle, thus eliciting greater peak blood flows. In contrast, EndEx bouts were expected to produce the largest relative increases in activity and increases in blood flow in the red gastrocnemius muscle fibers. Finally, we expected less effect on arteries perfusing the slow soleus muscle fibers, because these fibers have relatively greater activity during posture maintenance; thus they have relatively smaller increases in activity during training bouts. Importantly, this study was designed to evaluate effects of these two different types of training in treatment of advanced insulin resistance and T2D. Thus treatment was initiated at 20 wk of age, an age at which the OLETF rat is known to be progressing from advanced insulin resistance to overt T2D (9, 19, 29).

METHODS

Animals

All animal protocols were approved by the University of Missouri Institutional Animal Care and Use Committee. Male OLETF rats (at 4 wk of age) were purchased from the Tokushima Research Institute, Otsuka Pharmaceutical (Tokushima, Japan). Rats were single housed, on a 12:12-h light-dark cycle, and provided water and standard rodent chow (Formulab 5008, Purina Mills, St. Louis, MO) ad libitum. Body weights and food intakes were recorded on a weekly basis.

Protocols

At 20 wk of age, OLETF rats were randomly assigned to one of three groups: 1) sedentary, 2) EndEx, and 3) IST. For EndEx, treadmill running duration and intensity were increased progressively over the first 4 wk to reach 60 min of treadmill running at 20 m/min at a 15% incline for the remaining 8 wk (1.2 km/day). For IST, six bouts of treadmill running, with 4.5-min rest periods, were progressively increased in duration and intensity over the first 5 wk to reach running speeds of 40 m/min at a 15% incline for 2.5 min/bout for the remaining 7 wk (0.6 km/day). Both EndEx and IST groups exercised for 5 days/wk.

At 32 wk of age, following an overnight fast and ~20–24 h from the last exercise bout, rats were anesthetized (pentobarbital sodium, 100 mg/kg) between 0800 and 0930, and body weight and percent body fat were determined. The soleus and gastrocnemius muscles were then harvested for arteriole isolation. Rats were then euthanized by exsanguination with blood samples obtained for analysis. Following exsanguination, heart weights were determined, and skeletal muscle samples were taken from the red and white portions of the vastus lateralis, snap frozen, and stored at −80°C until processed. The red and white portions of the vastus lateralis were determined visually and harvested from the deep and superficial portions of the muscle, respectively. Citrate synthase activity was measured from whole muscle homogenate of the vastus lateralis using the spectrophotometric method of Srere (39). The vastus lateralis was chosen for citrate synthase analysis as the muscle tissues of the contralateral limb were dedicated to another independent study.

Body Composition

On the day of the experiments, body mass was measured to the nearest 0.01 g, and, following anesthetization, body composition was determined using rodent calibrated, dual-energy X-ray absorptiometry (Hologic QDR-1000).

Glycosylated Hemoglobin (HbA1c)

Whole blood was collected in EDTA-coated tubes for HbA1c analysis by boronate-affinity HPLC (Primus Diagnostics, Kansas City, MO).

Isolation of Skeletal Muscle Arteries/Arterioles

Gastrocnemius feed arteries (GFA), soleus feed arteries (SFA), second-order arterioles from the red portion of the gastrocnemius (G2A-R), and second-order arterioles from the white portion of the gastrocnemius (G2A-W) were isolated, as previously described (42), and placed in ice-cold MOPS-buffered physiological saline solution (PSS) containing (in mM) 145.0 NaCl, 4.7 KCl, 2.0 CaCl2, 1.17 MgSO4, 1.2 NaH2PO4, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA, and 25.0 MOPS at pH 7.4. Vasomotor responses were determined in one GFA, SFA, G2A-R, and G2A-W per animal.

Determination of Vasomotor Responses

Following isolation, vessels were cannulated on glass micropipettes, secured at each end with 9–0 ophthalmic suture, and attached to separate hydrostatic pressure reservoirs containing PSS plus albumin (1 g/100 ml) that were adjusted to achieve approximate in vivo intraluminal pressure: 90 and 67 cmH2O for GFA/SFA and G2A-R/G2A-W, respectively (1, 41). Vessels were treated with 80 mM KCl to verify viability, washed with PSS, and allowed to equilibrate for 1 h at 37°C. Vessels were visualized on an inverted microscope with luminal diameters quantified using calibrated video calipers. Following equilibration, arteries were preconstricted with phenylephrine (PE) to achieve ≥30% tone for examination of vasomotor responses.

Endothelium-dependent dilation (EDD) was determined in response to ACh (1 nM–100 μM) for each vessel. Next, vasomotor responses in insulin (1–1,000 μU/ml) were performed. To evaluate the role of endothelin-1 (ET-1) in the vasomotor response to insulin, vessels were incubated for 20 min with tezosentan (3 μM), a non-selective ETA- and ETB-receptor antagonist, and vasoreactivity to insu-
lin (1–1,000 μIU/ml) was reassessed. For insulin curves, serial doses were given in 10-min intervals, with vessel diameters recorded immediately before the introduction of the next dose of insulin. Following insulin curves, endothelium-independent dilation of vessels to sodium nitroprusside (SNP; 1 nM–100 μM) was also determined by serial doses at 3-min intervals. Finally, the PSS bath was replaced with Ca²⁺-free PSS to determine maximal passive diameter. Between each curve, vessel baths were washed with fresh PSS and equilibrated for 30 min.

To determine the relative contribution of the endothelium in the observed vasoreactivity to insulin, we evaluated the vasomotor response to insulin and insulin in the presence of tezosentan (as described above) in separate SFAs in which the endothelium was denuded (n = 5). The SFA was chosen for these experiments due to the substantial vasoreactivity observed in previous experiments. Vessels were denuded by passing air bubbles through the lumen and subsequently confirming the integrity of vascular smooth muscle cell function with 80 mM KCl.

Drugs and Solutions

All drugs and solutions were obtained from Sigma (St. Louis, MO), except albumin (USB, Cleveland, OH). PSS solutions and drugs were prepared before the study and frozen, and aliquots were thawed each day. All drug solutions were prepared in PSS, except insulin, which was prepared in PSS with albumin (1 g/100 ml). SNP was prepared fresh and protected from light on each day of the experiments.

Statistical Considerations

Data are presented as means ± SE. Dilator responses are presented as percent maximal dilation, calculated as: \[\frac{(D_d - D_b)}{(D_{max} - D_b)}\]·100, where \(D_d\) is diameter after drug intervention, \(D_b\) is baseline diameter, and \(D_{max}\) is maximal passive diameter. Percent ET-1 contribution in vasomotor response to insulin, at each dose, was calculated as follows: percent maximal dilation to insulin in the presence of tezosentan minus percent maximal dilation to insulin alone. Differences in animal and vessel characteristics were determined using a one-way ANOVA with a Tukey post hoc analysis. A P value of 0.05 was considered significant. Significant differences in citrate synthase activity were determined using a two-way ANOVA. When a significant group × fiber-type interaction was observed, the effect of group in each fiber type was determined using adjustment for multiple comparisons. To maintain a family-wise error rate, \(\alpha\) was adjusted for three comparisons at each drug dose (0.05/3 = 0.0167). Significant differences in vasomotor responses were determined using a mixed-design repeated-measures ANOVA. When a significant group × drug dose interaction was found, the effect of group at each drug dose was determined using adjustment for multiple comparisons (\(\alpha = 0.0167\)). Statistical analysis was performed using IBM SPSS Statistics 19 for Windows (Chicago, IL).

RESULTS

Animal Characteristics

EndEx and IST intervention significantly attenuated increases in body weight, %body fat, and HbA1c compared with sedentary animals (Fig. 1, A, D, and E). Furthermore, EndEx and IST groups had significantly higher heart weight-to-body weight ratios (Fig. 1C), while there was no difference in heart weight between groups (Fig. 1B). Although there was no difference between IST and EndEx groups in body weight and HbA1c, %body fat was significantly lower in EndEx animals compared with IST and Sed groups (Fig. 1D). Significant differences in heart weight, %body fat, and HbA1c between groups were observed using one-way ANOVA and Tukey post hoc analysis.

Fig. 1. Descriptive characteristics, glycemic control (HbA1c), and vastus lateralis (VL) citrate synthase (CS) activity in 32-wk-old sedentary (Sed; n = 12), endurance-trained (EndEx; n = 12), and interval sprint-trained (IST; n = 12) Otsuka Long-Evans Tokushima fatty (OLETF) rats. HW/BW, heart weight to body weight ratio. Values are means ± SE. a,b,c Values with different letter superscripts denote significant between-group differences (\(P < 0.05\)).
compared with IST (Fig. 1D). Food intake averaged over the course of intervention (weeks 20–32) was significantly greater in sedentary animals than EndEx and IST animals (32.9 ± 0.4 vs. 28.2 ± 0.4 and 27.6 ± 0.6 g/day, respectively; *P < 0.05). However, food intake was not different between EndEx and IST. There was a main effect of training as both EndEx and IST increased citrate synthase activity in the red and white portions of the vastus lateralis compared with sedentary animals (Fig. 1F). In addition, in the white portion of the vastus lateralis, citrate synthase activity following IST was significantly greater than EndEx. Together, these data suggest that IST did selectively recruit a greater proportion of white skeletal muscle tissue during exercise training.

**Vessel Characteristics**

Maximal passive diameters and percent preconstriction relative to maximal passive diameter are presented in Table 1. Within vessel, there were no significant differences among groups for maximal diameter or degree of preconstriction before any of the vessel response experiments.

**Table 1. Passive diameter and preconstriction percentage**

<table>
<thead>
<tr>
<th></th>
<th>Passive Diameter, μm</th>
<th>Preconstriction, %</th>
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<th></th>
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<tbody>
<tr>
<td></td>
<td>N</td>
<td>ACh</td>
<td>Ins</td>
<td>Ins + Tezo</td>
<td>SNP</td>
</tr>
<tr>
<td>GFA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sed</td>
<td>10</td>
<td>306.3 ± 10.6</td>
<td>36.9 ± 2.3</td>
<td>40.7 ± 3.6</td>
<td>41.0 ± 3.8</td>
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<tr>
<td>EndEx</td>
<td>11</td>
<td>311.6 ± 9.9</td>
<td>44.1 ± 1.9</td>
<td>46.9 ± 3.3</td>
<td>47.5 ± 3.4</td>
</tr>
<tr>
<td>IST</td>
<td>12</td>
<td>318.1 ± 13.5</td>
<td>40.0 ± 2.0</td>
<td>37.2 ± 2.0</td>
<td>43.9 ± 2.6</td>
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<tr>
<td>SFA</td>
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<tr>
<td>Sed</td>
<td>13</td>
<td>209.0 ± 7.1</td>
<td>62.8 ± 3.4</td>
<td>54.0 ± 2.8</td>
<td>49.0 ± 4.2</td>
</tr>
<tr>
<td>EndEx</td>
<td>9</td>
<td>218.9 ± 10.7</td>
<td>60.4 ± 5.6</td>
<td>55.8 ± 4.0</td>
<td>52.5 ± 4.6</td>
</tr>
<tr>
<td>IST</td>
<td>12</td>
<td>202.5 ± 9.7</td>
<td>64.4 ± 2.3</td>
<td>51.4 ± 2.8</td>
<td>51.2 ± 3.5</td>
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<tr>
<td>G2A-R</td>
<td></td>
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<tr>
<td>Sed</td>
<td>13</td>
<td>196.9 ± 10.2</td>
<td>36.8 ± 2.5</td>
<td>45.6 ± 3.4</td>
<td>39.1 ± 3.3</td>
</tr>
<tr>
<td>EndEx</td>
<td>11</td>
<td>188.5 ± 8.7</td>
<td>38.8 ± 2.8</td>
<td>42.0 ± 2.7</td>
<td>39.5 ± 3.5</td>
</tr>
<tr>
<td>IST</td>
<td>11</td>
<td>189.0 ± 10.6</td>
<td>46.5 ± 4.1</td>
<td>48.7 ± 2.6</td>
<td>52.8 ± 2.9</td>
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<tr>
<td>G2A-W</td>
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<tr>
<td>Sed</td>
<td>13</td>
<td>189.3 ± 12.3</td>
<td>33.0 ± 2.1</td>
<td>34.0 ± 2.0</td>
<td>39.0 ± 3.4</td>
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<tr>
<td>EndEx</td>
<td>12</td>
<td>165.9 ± 7.2</td>
<td>43.4 ± 3.4</td>
<td>42.8 ± 3.0</td>
<td>43.5 ± 4.3</td>
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<tr>
<td>IST</td>
<td>12</td>
<td>186.5 ± 21.6</td>
<td>36.5 ± 3.2</td>
<td>39.3 ± 4.6</td>
<td>38.0 ± 4.4</td>
</tr>
</tbody>
</table>

Values are means ± SE; N, no. of rats. ACh, acetylcholine; Ins, insulin; Tezo, tezosentan; SNP, sodium nitroprusside; GFA, gastrocnemius feed artery; SFA, soleus feed artery; G2A-R, second-order arterioles perfusing the red portion of the gastrocnemius; G2A-W, second-order arterioles perfusing the white portion of the gastrocnemius; Sed, sedentary; EndEx, endurance exercise; IST, interval sprint training.

**Table 2. Sensitivity and maximal dilatory response to acetylcholine and sodium nitroprusside**

<table>
<thead>
<tr>
<th></th>
<th>ACh</th>
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<tr>
<td></td>
<td>Sed</td>
<td>EndEx</td>
<td>IST</td>
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<tr>
<td>GFA</td>
<td></td>
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<tr>
<td>Maximum dilation, %</td>
<td>41.3 ± 6.9</td>
<td>57.3 ± 8.9</td>
<td>70.2 ± 7.6*</td>
<td>74.7 ± 5.1</td>
</tr>
<tr>
<td>EC50, −log M</td>
<td>−4.9 ± 0.5</td>
<td>−5.7 ± 0.5</td>
<td>−6.1 ± 0.5</td>
<td>−6.1 ± 0.4</td>
</tr>
<tr>
<td>SFA</td>
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<tr>
<td>Maximum dilation, %</td>
<td>93.6 ± 1.8</td>
<td>94.8 ± 1.5</td>
<td>95.6 ± 0.9</td>
<td>70.8 ± 5.5</td>
</tr>
<tr>
<td>EC50, −log M</td>
<td>−7.5 ± 0.2</td>
<td>−7.3 ± 0.1</td>
<td>−7.5 ± 0.1</td>
<td>−5.2 ± 0.2</td>
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<tr>
<td>G2A-R</td>
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</tr>
<tr>
<td>Maximum dilation, %</td>
<td>58.5 ± 8.6</td>
<td>85.5 ± 5.4*</td>
<td>83.7 ± 4.0*</td>
<td>62.8 ± 6.2</td>
</tr>
<tr>
<td>EC50, −log M</td>
<td>−5.0 ± 0.4</td>
<td>−6.7 ± 0.3*</td>
<td>−6.7 ± 0.2*</td>
<td>−5.5 ± 0.4</td>
</tr>
<tr>
<td>G2A-W</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum dilation, %</td>
<td>45.4 ± 4.9</td>
<td>71.8 ± 6.9**</td>
<td>47.3 ± 6.0</td>
<td>65.4 ± 5.2</td>
</tr>
<tr>
<td>EC50, −log M</td>
<td>−4.3 ± 0.2</td>
<td>−5.6 ± 0.3**</td>
<td>−4.6 ± 0.3</td>
<td>−5.6 ± 0.4</td>
</tr>
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</table>

Values are means ± SE. EC50, half-maximal effective concentration (sensitivity); maximum dilation, maximal percent possible dilation at any dose. *P < 0.05 vs. Sed. †*P < 0.05 vs. IST.
Insulin following exercise training were only detected in the G2A-R and G2A-W. In the G2A-R, at 10–1,000 μIU/ml concentrations of insulin, vasodilation was significantly greater in the EndEx group compared with IST (Fig. 3C); however, there was no difference between EndEx and sedentary groups at any concentration of insulin. These group differences in the G2A-R were abolished with tezosentan (Fig. 3G). In the G2A-W, vasoreactivity to insulin was minimal, and there were no differences between groups. However, in the presence of tezosentan, insulin induced dilation in all groups. This vasodilation was greater in IST animals compared with EndEx at 100 and 1,000 μIU/ml concentrations of insulin (Fig. 3H).

In denuded SFA experiments, vasoreactivity to insulin was attenuated by ~85% in the SFA. Specifically, the mean endothelial contribution for all doses of insulin with and without tezosentan was 79 and 88%, respectively.

Role of ET-1 in vasomotor response to insulin. There were no differences in the contribution of ET-1 constriction in the vasomotor response to insulin between any group for GFAs (Fig. 4A) and SFAs (Fig. 4B). In the G2A-R, there was significantly less contribution of ET-1 to the vasomotor response to insulin in the EndEx animals compared with IST animals at 10–1,000 μIU/ml concentrations of insulin (Fig. 4C). In the G2A-W, there was a significantly greater contribution of ET-1 in the vasomotor response to insulin in the IST animals compared with sedentary (at 1 μIU/ml) and EndEx (at 100 μIU/ml; Fig. 4D).

Effects of exercise on endothelium-independent vasodilation. Sensitivity and maximal dilatory response to SNP are presented in Table 2. Dilation to SNP was similar among treatment groups in the GFA, G2A-R, and G2A-W (Fig. 4, A, C, and D). However, in the SFA, vasodilation in the IST group was significantly greater than in the sedentary group at 1 and 10 nM concentrations of SNP and greater than in the EndEx group at 10 nM SNP (Fig. 5B). However, there were no differences between groups in sensitivity to SNP in the SFAs (Table 2).

**DISCUSSION**

Using 20-wk-old OLETF rats, which already have insulin resistance and obesity due to hyperphagia and inactivity (9, 19) and are close to developing T2D, we examined treatment with two different exercise programs that concentrate increased activity during exercise to different types of motor units (i.e., EndEx and IST) on regional adaptations in microvascular EDD, vasomotor responses to insulin, and endothelium-independent dilation in resistance arteries of the hindlimb skeletal muscle. The principle findings are that, in the OLETF rat model, 1) both EndEx and IST increased EDD in second-order arterioles of the red portions of the gastrocnemius muscle, whereas only EndEx was found to increase EDD in second-order arterioles of the white portion of the gastrocnemius; 2) neither EndEx nor IST increased insulin-induced vasodilation to insulin alone, in any vessel, compared with sedentary animals; and 3) there was significantly less ET-1 contribution in the vasomotor response to insulin following EndEx in second-order arterioles of the gastrocnemius. Importantly, these microvascular effects of EndEx and IST were observed in conjunction with significant training-related improvements in glycemic control compared with sedentary animals, as HbA1c
values were 21.2 and 22.5% lower in EndEx and IST treated animals, respectively.

The present study confirms that feed arteries perfusing the soleus demonstrate resistance to the development of endothelial dysfunction in the OLETF rat, a finding likely due to relatively higher soleus muscle blood flow and muscle fiber recruitment during nonexercise activity (i.e., posture and standing) (25). Indeed, soleus blood flow in the rat does not increase significantly from preexercise values (measured with the rats maintaining posture on the treadmill) at treadmill speeds of up to 75 m/min (24). A recent investigation in our laboratory demonstrated that voluntary wheel running beginning at 4 wk of age prevented vascular dysfunction in the GFA of OLETF rats (7). In contrast, in the present study, where endurance treadmill exercise was used as a treatment of advanced insulin resistance, EndEx did not significantly alter EDD to ACh in the GFA compared with sedentary animals (main effect, \( P = 0.284 \)). It is possible that exercise did not improve EDD in the GFA in this study because the mode of activity used is different than wheel running. Since our goal was to mimic training regimens commonly prescribed for the treatment of T2D in humans and to control muscle fiber recruitment patterns during exercise bouts, we chose to use treadmill training. In our previous investigation using voluntary wheel running, animals ran \( \sim 5-10 \) km/day, which was dispersed throughout the dark cycle. In the EndEx protocol used herein, animals ran \( \sim 1.2 \) km/day at a single point in the day. Therefore, differences in the absolute volume of running distance between studies could contribute to discrepancies in the magnitude of adaptation observed. In our view, the most important difference between the present study and the previous study (7) is that exercise was used as a treatment, and thus it began when OLETF animals

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**Fig. 3.** Vascular reactivity to insulin alone (A–D) and to insulin in the presence of the nonselective endothelin-1 (ET-1) receptor antagonist tezosentan (E–H) across all doses of insulin in 32-wk-old Sed, EndEx, and IST OLETF rats. A and E: GFA. B and F: SFA. C and G: G2A-R. D and H: G2A-W. Values are expressed as mean percent possible dilation \( \pm \) SE. Within each group and vessel, \( n = 9–12 \). \( \delta \) EndEx vs. IST (\( P < 0.0167 \)).
were 20 wk of age. In our laboratory’s previous investigation, animals were given access to running wheels at 4 wk of age and killed for study at 20 wk. Therefore, the ability of exercise to enhance EDD to ACh in the OLETF rat model may be significantly impacted by whether it is used in the context of prevention or treatment of vascular complications of T2D. We hypothesized that IST, due to greater fast-twitch glycolytic and fast-twitch oxidative, glycolytic fiber-type recruitment, and the concomitant increase in blood flow would elicit the greatest effect on EDD in the feed artery and second-order arterioles of the gastrocnemius. Indeed, considering the incline used in the training protocols for the present study, if change in skeletal muscle blood flow is approximated by comparison to higher running speeds with no incline (e.g., EndEx and IST training is similar to 30 and 75 m/min on flat ground, respectively), one would expect significant increases from preexercise blood flow to the G2A-R during EndEx (−211%) and to the G2A-R (−487%) and G2A-W (−117%) during IST (24). In the GFA, although the EC50 and single drug dose analyses did not reach our Bonferroni-adjusted α of 0.0167, there was a main effect (P = 0.046) and significantly higher maximal EDD to ACh in IST rats compared with sedentary animals. However, IST was not found to be more effective than EndEx (P = 0.315). Interestingly, although EndEx and IST were both effective in improving EDD to ACh in the G2A-R, only EndEx

Fig. 4. ET-1-dependent component of vascular reactivity to insulin (difference with and without tezosentan) in GFAs (A), SFAs (B), G2A-R (C), and G2A-W (D) of 32-wk-old Sed, EndEx, and IST OLETF rats. Values are expressed as mean difference ± SE. Within each group and vessel, n = 9–12. φSed vs. IST (P < 0.0167). △EndEx vs. IST (P < 0.0167).

Fig. 5. Concentration-response curves in 32-wk-old Sed, EndEx, and IST OLETF rats to sodium nitroprusside (SNP) in GFAs (A), SFAs (B), G2A-R (C), and G2A-W (D). Within each group and vessel, n = 9–12. φSed vs. IST (P < 0.0167); △EndEx vs. IST (P < 0.0167). Values are means ± SE.
had an effect in the G2A-W. It is reasonable to conclude that, with the higher intensity IST training, there would exist at least the same increase in blood flow to the white portion of the gastrocnemius as during EndEx, be it for a shorter total duration each training day. Moreover, although we measured citrate synthase in the vastus lateralis, our data indicate that there was a significant training effect in both the “red” and “white” skeletal muscle following both EndEx and IST, but greater “white” recruitment with IST. Therefore, the greater adaptive response in EDD to ACh in the G2A-W following EndEx was surprising. This may be due, in part, to the nature of the second-order arterioles. In an effort to isolate second-order arterioles perfusing white and red tissue, second-order branches that perfused the lateral and medial aspects of the medial head of the gastrocnemius muscle, respectively, were harvested for analysis. While the lateral and medial portions of the gastrocnemius are composed of majority white and red fibers, respectively, the tissue, as a whole, is composed of “mixed” fiber types. Furthermore, the nature of the exercise-training stimulus (continuous vs. interval) and the resultant increase in blood flow shear stress, with upregulation of the nitric oxide (NO) signaling pathway (26), during EndEx and IST may play a significant role in the differential adaptive response in EDD to ACh.

Insulin-mediated vasoreactivity is determined, in part, by the balance between vasodilation produced through endothelium-derived NO via phosphatidylinositol 3-kinase (PI3K)/Akt signaling and vasoconstriction mediated by ET-1 via mitogen-activated protein kinase signaling (20). In insulin resistance states, there is a shift toward greater stimulation of the mitogen-activated protein kinase pathway and inhibition of the PI3K pathway, which is compounded by the reciprocal relationship between the two (33). Our group has previously demonstrated that physical activity (i.e., wheel running) can prevent impairments in insulin-induced vasodilation in the G2A-W of OLETF rats, independent of changes in adiposity (31). In that study, OLETF rats were equipped with voluntary running wheels at 4 wk of age to evaluate daily physical activity as an obesity and insulin resistance prevention strategy. However, the ability of treadmill exercise, in line with what may be prescribed for treatment of insulin resistance and T2D in humans, to improve microvascular insulin sensitivity has not been elucidated. In the present study, neither EndEx nor IST led to enhancements in vasoreactivity to insulin alone, in any vessel, compared with sedentary animals. However, insulin-induced ET-1 activation was significantly less in EndEx than IST in both the G2A-R and G2A-W. One would expect, in the adaptation to EndEx in the G2A-R and G2A-W, that there would be a concurrent increase in the vascular PI3K/endothelial NO synthase/Akt vasodilator pathway (i.e., NO production). However, greater vasodilation to insulin alone was only observed in the G2A-R of EndEx animals compared with IST. Interestingly, following IST, in the G2A-W, there was no net vasomotor response in the presence of insulin alone, but significantly greater dilation than EndEx in the presence of an ET-1 antagonist. Based on these observations, it appears that IST upregulated both the NO and ET-1 arms of vascular insulin signaling pathways in the G2A-W.

In denuded vessels, the vasoreactivity to insulin alone, and insulin in the presence of tezosentan, was mostly abolished (~85%). Therefore, we are confident that vasoreactivity to insulin, and the differential response following IST and EndEx training, are primarily due to differences in endothelial adaptations. Our data also suggest that the observed differences between IST and EndEx in insulin-induced vasodilation in the presence of tezosentan are primarily mediated by NO. Indeed, our group has previously shown that group differences in insulin-induced vasoreactivity between voluntary wheel-running OLETF rats and their sedentary counterparts in the G2A-W are abolished in the presence of the NO synthase blocker N^6-nitro-L-arginine methyl ester (31).

In the present study, exercise intervention commenced at 20 wk of age, as our laboratory has previously demonstrated that the OLETF rat becomes hyperglycemic and hyperinsulinemic at this age (9, 29, 35, 36). Moreover, our laboratory’s previous investigations have shown that HbA1c values in sedentary OLETF rats progress from ~5.3 to 8.8% from 20 to 40 wk of age (29, 36). In the present study, at 32 wk of age, the sedentary OLETFs demonstrated considerable progression of disease, as HbA1c values were considerably higher (6.84 ± 0.23%) than those typically seen at 20 wk of age. For comparison, these HbA1c values exceed the 6.5% threshold for clinical diagnosis of T2D in humans (2). Both EndEx and IST treatment strategies were equally effective in attenuating the deterioration in glycemic control (5.39 ± 0.06 and 5.30 ± 0.14% in EndEx and IST, respectively). However, HbA1c values did not reach levels typically observed by our group in lean, wild-type rats whose values range from ~4.6 to 4.8% between the ages of 20 and 40 wk (29, 36). It could be hypothesized that IST would have a greater benefit on glycemic control due to spatially focused improvements in glucose uptake, endothelial function, and insulin-induced vasodilation in the “white” and mixed muscle. However, existing literature in humans suggests that both IST and EndEx can improve glycemic control (13). The similar outcomes in glycemic control following EndEx and IST treatment may be due, in part, to relatively equal effects on glucose clearance in different areas of the skeletal muscle. In addition, body weight gain and fat mass were suppressed to a similar degree in both the EndEx and IST rats compared with sedentary rats in the present study, a finding that could have contributed to the observed adaptations (11). However, the EndEx and IST groups still demonstrated differential adaptations in skeletal muscle arteriolar function, indicating that alterations in skeletal muscle arteriolar vasoreactivity to insulin may occur independent of changes in long-term measures of glycemic control, such as HbA1c. It is possible that insulin-induced glucose uptake is altered locally at sites where microvascular adaptation is observed, but this should be determined in future investigations.

In summary, both EndEx and IST are effective treatment strategies in ameliorating the decline in glycemic control observed in hyperphagic, obese OLETF rats. In addition, both exercise treatments improved vascular function, primarily downstream of the GFAs. While both exercise treatments improved EDD to ACh in the G2A-R, only EndEx enhanced EDD to ACh in the G2A-W. Moreover, although neither EndEx nor IST improved vasomotor response to insulin compared with sedentary animals, there was significant heterogeneity in the adaptive responses of the vascular insulin signaling pathways that primarily reflected differences between second-order arterioles in training-induced changes in insulin-mediated ET-1 activation. Overall, this study provides evidence that...
treatment of advanced insulin resistance in the OLETF rat with exercise paradigms that elicit diverse motor recruitment patterns produce differential adaptive responses not only to EDD, but also to regulation of the complex vascular actions of insulin.

ACKNOWLEDGMENTS

The authors are grateful to Pam Thorne and Grace Meers for excellent technical assistance on this project. We also thank Eric Gibson, Brittany Muller, Kelcie Tacchi, Matt Brielmaier, and Nicholas Fleming for all of their hard work as animal trainers. This work was partially supported with resources and the use of facilities at the Harry S. Truman Memorial Veterans Hospital in Columbia, MO.

GRANTS

This work was supported by National Institutes of Health (NIH) T32-AR048523 (J. S. Martin and N. T. Jenkins), American Heart Association 11POST5080002 (J. Padilla), VA-CDA-IK2 BX001299-01 (R. S. Rector), and NIH RO1HL063688 (to M. H. Laughlin).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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