Adipose tissue and vascular phenotypic modulation by voluntary physical activity and dietary restriction in obese insulin-resistant OLETF rats

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1Biomedical Sciences, University of Missouri, Columbia, Missouri; 2Kinesiology, University of Georgia, Athens, Georgia; 3Nutrition and Exercise Physiology, University of Missouri, Columbia, Missouri; 4Internal Medicine-Division of Gastroenterology and Hepatology, University of Missouri, Columbia, Missouri; 5Research Service, Harry S. Truman Virginia Memorial Medical Center, Columbia, Missouri; 6Dalton Cardiovascular Research Center, University of Missouri, Columbia, Missouri; 7Medical Pharmacology and Physiology, University of Missouri, Columbia, Missouri; and 8Child Health, University of Missouri, Columbia, Missouri

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Crissey JM, Jenkins NT, Lansford KA, Thorne PK, Bayless DS, Vieira-Potter VJ, Rector RS, Thyfault JP, Laughlin MH, Padilla J. Adipose tissue and vascular phenotypic modulation by voluntary physical activity and dietary restriction in obese insulin-resistant OLETF rats. Am J Physiol Regul Integr Comp Physiol 306: R596–R606, 2014. First published February 12, 2014; doi:10.1152/ajpregu.00493.2013.—Adipose tissue (AT)-derived cytokines are proposed to contribute to obesity-associated vascular insulin resistance. We tested the hypothesis that voluntary physical activity and diet restriction-induced maintenance of body weight would both result in decreased AT inflammation and concomitant improvements in insulin-stimulated vascular relaxation in the hyperphagic, obese Otsuka Long-Evans Tokushima fatty (OLETF) rat. Rats (aged 12 wk) were randomly assigned to sedentary (SED; n = 10), wheel running (WR; n = 10), or diet restriction (DR; n = 10; fed 70% of SED) for 8 wk. WR and DR rats exhibited markedly lower adiposity (7.1 ± 0.4 and 15.7 ± 1.1% body fat, respectively) relative to SED (27 ± 1.2% body fat), as well as improved blood lipid profiles and systemic markers of insulin resistance. Reduced adiposity in both WR and DR was associated with decreased AT mRNA expression of inflammatory genes (e.g., MCP-1, TNF-α, and IL-6) and markers of immune cell infiltration (e.g., CD8, CD11c, and F4/80). The extent of these effects were most pronounced in visceral AT compared with subcutaneous and periaortic AT. Markers of inflammation in brown AT were upregulated with WR but not DR. In periaortic AT, WR- and DR-induced reductions in expression and secretion of cytokines were accompanied with a more atheroprotective gene expression profile in the adjacent aortic wall. WR, but not DR, resulted in greater insulin-stimulated relaxation in the aorta; an effect that was, in part, mediated by a decrease in insulin-induced endothelin-1 activation in WR aorta. Collectively, we show in OLETF rats that lower adiposity leads to less AT and aortic inflammation, as well as an exercise-specific improvement in insulin-stimulated vasorelaxation.

lin resistance, Type 2 diabetes, and cardiovascular disease (21). A critical link between obesity and its associated metabolic and cardiovascular diseases is thought to be chronic low-grade systemic inflammation (21). In this regard, recent studies implicate adipose tissue (AT) as a local and systemic source of inflammatory cytokines that may be involved in the instigation of vascular insulin resistance and atherosclerosis associated with obesity (12, 13, 19, 20, 31, 33–36, 45, 46, 51, 55, 56). Indeed, excessive lipid accumulation and enlargement of adipocytes in obesity are associated with infiltration of immune cells into AT, contributing to AT inflammation and subsequent secretion of proinflammatory cytokines (52). A deeper understanding of the influence of lifestyle modifications on AT inflammation and vascular insulin resistance may lead to more effective strategies aimed at prevention and treatment of obesity-related metabolic and cardiovascular diseases.

Accordingly, we tested the hypothesis that treatment with increased voluntary physical activity or dietary restriction-induced maintenance of body weight would result in decreased AT inflammation and concomitant improvements in vasomotor reactivity to insulin in obese, insulin-resistant rats. Given the growing appreciation for phenotypic and functional heterogeneity among AT depots, including visceral, subcutaneous, brown, and perivascular adipose tissue (16, 17, 42, 48), we also reasoned that the extent of the effects of physical activity and diet restriction on AT inflammation would be AT depot-specific. Furthermore, we tested the hypothesis that reduced AT expression and secretion of cytokines caused by physical activity or dietary restriction would be accompanied by a less proatherogenic vascular phenotype and enhanced insulin-stimulated vascular relaxation.

METHODS

Animals. All animal protocols were approved by the University of Missouri Institutional Animal Care and Use Committee. Male Otsuka Long-Evans Tokushima fatty (OLETF) rats were obtained at 4 wk of age (Japan SLC, 3371–8; Kotoh-Cho, Hamamatsu, Shizuoka, Japan) and housed individually in cages maintained at temperature-controlled (21°C) animal quarters with lights on from 0600 to 1800 and dark from 1800 to 0600. At 12 wk, rats were randomized to one of the following three groups: 1) sedentary (SED; n = 10); 2) voluntary wheel running (WR; n = 10); or 3) sedentary + diet restriction (DR, fed 70% of ad libitum-fed SED

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MORE THAN ONE-THIRD OF AMERICANS are obese (38), and the causes underlying the obesity epidemic appear to be largely related to physical inactivity and overnutrition, a set of behaviors increasingly prevalent in our society (5–7, 11, 57). Cumulative evidence indicates that obesity is an important contributor to the development of whole body insu-
The cages of all groups wheels of the WR group were locked, and food was removed from euthanized by exsanguination in full compliance with the American Veterinary Medical Association Guidelines on Euthanasia. The wheels of the WR group were locked, and food was removed from the cages of all groups ~14 h before the rats were euthanized.

**Body composition and blood parameters.** On the day of the experiments, body mass was measured to the nearest 0.01 g and, following anesthetization, body composition was determined using a dual-energy X-ray absorptiometry instrument (Hologic QDR-1000) calibrated for rodents. In addition, retroperitoneal, epididymal, and omental AT weights were measured to the nearest 0.01 g. Plasma samples were prepared by centrifugation and stored at ~80°C until analysis. Glucose, cholesterol, triglycerides, and nonesterified fatty acids (NEFA) assays were performed by a commercial laboratory (Comparative Clinical Pathology Services, Columbia, MO) on an Olympus AU680 automated chemistry analyzer (Beckman-Coulter, Brea, CA), using commercially available assays, according to the manufacturer’s guidelines. Plasma insulin concentrations were determined using a commercially available, rat-specific ELISA (Alpco Diagnostics, Salem, NH). In addition, plasma and periaortic AT-conditioned medium samples were available, rat-specific ELISA (Alpco Diagnostics, Salem, NH). Insulin concentrations were determined using a commercially available, rat-specific ELISA (Alpco Diagnostics, Salem, NH).

**Cytokine secretion from periaortic AT.** A portion of perivasculat AT surrounding the thoracic aorta was incubated in Medium 199 at pH 7.4 for 24 h (100 mg of AT/300 μl) under standard culture conditions (37°C, 5% CO₂) to obtain the corresponding secretomes (SED, WR, and DR) (2). After 24 h of incubation, the conditioned media from the AT explants were stored at ~80°C until analysis.

**RNA extraction and real-time PCR.** AT and aortic samples were homogenized in TRizol solution using a tissue homogenizer (TissueLyser LT, Qiagen, Valencia, CA). Total RNA was isolated using the Qiagen’s RNeasy Lipid Tissue Kit and assayed using a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE) to assess purity and concentration. First-strand cDNA was synthesized from total RNA using the high-capacity cDNA reverse transcription kit (Applied Biosystems, Carlsbad, CA). Quantitative real-time PCR was performed as previously described (40–42) using the CFX Connect Real-Time PCR Detection System (Bio-Rad, Hercules, CA).

**Tissue sampling.** Perivasular AT surrounding the thoracic aorta, retroperitoneal white AT, inguinal subcutaneous white AT, and interscapular brown AT were quickly excised from the anesthetized rat. For each fat depot, a portion was flash frozen and kept at ~80°C for 18 h before the rats were euthanized.

**Tissue sampling.** Perivasular AT surrounding the thoracic aorta, retroperitoneal white AT, inguinal subcutaneous white AT, and interscapular brown AT were quickly excised from the anesthetized rat. For each fat depot, a portion was flash frozen and kept at ~80°C for examination of gene expression, and a portion was fixed in neutral-buffered 10% formalin for histology analysis. A portion of perivasular AT surrounding the thoracic aorta was used for in vitro assessment of cytokine secretion as described below. A segment of the thoracic aorta cleaned of perivasculat AT and excess adventitia was sectioned into 2-mm rings in cold Krebs for subsequent assessment of vasomotor function. In addition, isolated thoracic aortic segments were kept in RNAlater (Ambion, Austin, TX) for 24 h at 4°C, and then removed from the RNAlater solution and stored at ~80°C until processing.

**Body composition and blood parameters.** On the day of the experiments, body mass was measured to the nearest 0.01 g and, following anesthetization, body composition was determined using a dual-energy X-ray absorptiometry instrument (Hologic QDR-1000) calibrated for rodents. In addition, retroperitoneal, epididymal, and omental AT weights were measured to the nearest 0.01 g. Plasma samples were prepared by centrifugation and stored at ~80°C until analysis. Glucose, cholesterol, triglycerides, and nonesterified fatty acids (NEFA) assays were performed by a commercial laboratory (Comparative Clinical Pathology Services, Columbia, MO) on an Olympus AU680 automated chemistry analyzer (Beckman-Coulter, Brea, CA), using commercially available assays, according to the manufacturer’s guidelines. Plasma insulin concentrations were determined using a commercially available, rat-specific ELISA (Alpco Diagnostics, Salem, NH). In addition, plasma and periaortic AT-conditioned medium samples were available, rat-specific ELISA (Alpco Diagnostics, Salem, NH). Insulin concentrations were determined using a commercially available, rat-specific ELISA (Alpco Diagnostics, Salem, NH).

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**Gene Forward Reverse**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
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<tr>
<td>18S</td>
<td>GCCGCATTAGAGCTGAAATTTCTTG</td>
<td>CATTCTGAGCAAATTTCTTTCG</td>
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<tr>
<td>Leptin</td>
<td>GACACCTTACAGAGGCCTCTGA</td>
<td>ACGAGGAGCGCCCTTCTTCA</td>
</tr>
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<td>MCP-1</td>
<td>CTGCCATGTCGGACTGGCTTAA</td>
<td>AGCGGAGCTGCCGCAGTATGAT</td>
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<td>TNF-α</td>
<td>AACCAGAGAGAGCCTGAAAT</td>
<td>TCCAGTCTGAGCTCAGAAGCC</td>
</tr>
<tr>
<td>IL-6</td>
<td>AGAGAGAGAGAGCCTGAAAT</td>
<td>TCCAGTCTGAGCTCAGAAGCC</td>
</tr>
<tr>
<td>E-Selectin</td>
<td>GCCATGTCGGACTGGCTTAA</td>
<td>AGCGGAGCTGCCGCAGTATGAT</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>CCGAGAAAACCTGGAGAAAGAAGAAAGC</td>
<td>CCGAGAAAACCTGGAGAAAGAAGAAAGC</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>CCAAGAGAGAGAGCCTGAAAT</td>
<td>TCCAGTCTGAGCTCAGAAGCC</td>
</tr>
<tr>
<td>PAI-1</td>
<td>AGTGGAGAGAGAGCCTGAAAT</td>
<td>TCCAGTCTGAGCTCAGAAGCC</td>
</tr>
<tr>
<td>CD4</td>
<td>ACCCGGAGAGAGCCTGAAAT</td>
<td>TCCAGTCTGAGCTCAGAAGCC</td>
</tr>
<tr>
<td>CD8</td>
<td>ACCCGGAGAGAGCCTGAAAT</td>
<td>TCCAGTCTGAGCTCAGAAGCC</td>
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<td>CD11c</td>
<td>ACCCGGAGAGAGCCTGAAAT</td>
<td>TCCAGTCTGAGCTCAGAAGCC</td>
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<tr>
<td>F4/80</td>
<td>GCCATGTCGGACTGGCTTAA</td>
<td>AGCGGAGCTGCCGCAGTATGAT</td>
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<tr>
<td>Foxp3</td>
<td>GCCATGTCGGACTGGCTTAA</td>
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<td>CHOP</td>
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<td>GRP78</td>
<td>GCCATGTCGGACTGGCTTAA</td>
<td>TCCAGTCTGAGCTCAGAAGCC</td>
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<td>p22phox</td>
<td>ACCCGGAGAGAGCCTGAAAT</td>
<td>TCCAGTCTGAGCTCAGAAGCC</td>
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<td>p47phox</td>
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<tr>
<td>UCP-1</td>
<td>GCCGTGCATTAGAGCTGAAAT</td>
<td>TCCAGTCTGAGCTCAGAAGCC</td>
</tr>
<tr>
<td>PPARGC-1-α</td>
<td>GCCGTGCATTAGAGCTGAAAT</td>
<td>TCCAGTCTGAGCTCAGAAGCC</td>
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</table>
Histology assessments. Formalin-fixed AT samples were processed through paraffin embedment, sectioned at 5 μm, stained with hematoxylin and eosin for morphometric determinations and anti-UCP-1 antibody produced in rabbit (1:1,200 dilution, U6382; Sigma, St. Louis, MO), as previously described (1, 25, 41, 42). Sections were examined using an Olympus BX60 photomicroscope (Olympus, Melville, NY) and photographed at 40 magnification using a Spot Insight digital camera (Diagnostic Instruments, Sterling Heights, MI). All procedures were performed by an investigator who was blinded to the experimental conditions.

Functional assessment of isolated aortic rings. A segment of the thoracic aorta, trimmed of fat and connective tissue, was sectioned into 2-mm rings in cold Krebs. Rings were then mounted on wire feet connected to isometric force transducers and submerged in 20-ml baths containing physiological Krebs solution maintained at 37°C for 1 h to allow for equilibration. Aortic rings were stretched to optimal

Table 2. Fasting plasma characteristics in sedentary, wheel running, and diet restriction OLETF rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>SED</th>
<th>WR</th>
<th>DR</th>
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<tbody>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>110.7 ± 4.4</td>
<td>69.7 ± 2.8*</td>
<td>78.0 ± 1.1*</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>46.4 ± 3.9</td>
<td>29.6 ± 1.8*</td>
<td>34.5 ± 0.8*</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>33.2 ± 0.7</td>
<td>29.9 ± 1.3*</td>
<td>29.1 ± 0.5*</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>155.0 ± 6.5</td>
<td>51.1 ± 2.7*</td>
<td>71.9 ± 4.0#</td>
</tr>
<tr>
<td>NEFA, mmol/l</td>
<td>0.57 ± 0.05</td>
<td>0.19 ± 0.01*</td>
<td>0.31 ± 0.03*#</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>30.8 ± 10.1</td>
<td>6.9 ± 0.7*</td>
<td>19.5 ± 3.2</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>311.6 ± 9.5</td>
<td>196.1 ± 4.9*</td>
<td>223.1 ± 10.6#</td>
</tr>
<tr>
<td>HOMA-IR index</td>
<td>24.1 ± 8.2</td>
<td>3.4 ± 0.4*</td>
<td>11.1 ± 2.2</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>260.1 ± 39.0</td>
<td>2.2 ± 0.3*</td>
<td>63.9 ± 24.7*</td>
</tr>
<tr>
<td>MCP-1, pg/ml</td>
<td>197.1 ± 8.5</td>
<td>144.3 ± 9.4*</td>
<td>194.4 ± 26.4#</td>
</tr>
<tr>
<td>TNF-α, pg/ml</td>
<td>6.5 ± 0.3</td>
<td>5.3 ± 0.5</td>
<td>6.6 ± 0.5#</td>
</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>204.4 ± 66.0</td>
<td>173.6 ± 56.7</td>
<td>194.5 ± 66.3</td>
</tr>
</tbody>
</table>

SED, sedentary; WR, wheel running; DR, diet restriction; LDL, low-density lipoprotein; HDL, high-density lipoprotein; NEFA, nonesterified fatty acids; HOMA-IR, homeostasis model assessment of insulin resistance; MCP-1, monocyte chemotactic protein-1; TNF-α, tumor necrosis factor alpha; IL-6, interleukin 6. *Significant difference (P < 0.05) from SED rats. #Significant difference (P < 0.05) from WR rats.

Fig. 1. Body composition and food intake in sedentary (SED), wheel running (WR), and diet restriction (DR) Otsuka Long-Evans Tokushima fatty (OLETF) rats. Values are expressed as means ± SE; n = 10/group. Body fat, heart weights, and fat pad weights were obtained at 20 wk (time of death). *Significant difference (P < 0.05) from SED rats. #Significant difference (P < 0.05) from WR rats.
length, which ranged from 130 to 140% of passive diameter. Vaso-reactivity was assessed with cumulative concentration-response curves of ACh ($10^{-10}$ to $10^{-4}$ M), insulin (10 to 10,000 μIU/ml), sodium-nitroprusside (SNP; $10^{-10}$ to $10^{-4}$ M), and endothelin-1 (ET-1; $10^{-10}$ to $10^{-7}$ M). A submaximal concentration of phenylephrine ($3 \times 10^{-7}$ M) was used to preconstrict all vessels prior to ACh, insulin, and SNP relaxation curves. The contribution of ET-1 in altering insulin-stimulated relaxation was assessed by incubating the rings with tezosentan (3 μM for 20 min), a nonselective ET-1 receptor blocker. For insulin, ACh, and SNP curves, relaxation at each concentration was measured and expressed as percent maximum relaxation, where 100% is equivalent to loss of all tension developed in response to phenylephrine (10).

**Statistical analysis.** To determine the differences among SED, WR, and DR groups on all dependent variables, a one-way ANOVA with multiple comparisons was performed followed by Fisher’s protected least significant difference as post hoc test. Dose-response curves from vasomotor function experiments were analyzed using a two-way (group × dose) ANOVA. All data are presented as means ± SE. For all statistical tests, the α-level was set at 0.05. All statistical analyses were performed with SPSS version 21.0.

**RESULTS**

As shown in Fig. 1, rats with access to running wheels increased daily running distance from week 12 to week 15 (~8 km/day), after which running distance gradually declined to ~6 km/day at 20 wk. These are similar voluntary running distances, as we have previously reported in this animal model (32, 50). WR and DR rats weighed less and had improved body composition profiles (e.g., lower total percent body fat and less

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Fig. 2. A: representative histology photographs (×40 magnification) of retroperitoneal adipose tissue (AT), subcutaneous AT, interscapular brown AT, and periaortic AT in sedentary (SED), wheel running (WR), and diet restriction (DR) Otsuka Long-Evans fatty (OLETF) rats. B: representative photographs (×40 magnification) of immunohistochemical staining (shown here in brown) for uncoupling protein-1 (UCP-1) in interscapular brown AT and periaortic AT from SED, WR, and DR OLETF rats.
visceral adipose tissue mass) compared with SED rats (all $P < 0.05$). As summarized in Table 2, compared with the SED group, WR and DR plasma had lower fasting total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, NEFAs, glucose, and leptin (all $P < 0.05$). In addition, WR had improved HOMA-IR, lower plasma insulin, and lower circulating MCP-1 compared with SED rats (all $P < 0.05$).

Figure 2A illustrates representative histological photographs of retroperitoneal AT, subcutaneous AT, interscapular brown AT, and periaortic AT. As shown, WR and DR had less lipid deposition in brown and periaortic AT, as well as decreased adipocyte size in white AT. Consistent with our previous report (42), a clear structural similarity between thoracic perivascular AT and interscapular brown AT was noted. As depicted in Fig. 2B, immunoreactivity of UCP-1 appears to be similar between brown AT and periaortic AT, thus again highlighting the phenotypic similarity between these two fat depots.

Retroperitoneal AT of WR and DR rats exhibited reduced expression of leptin, MCP-1, TNF-α, IL-6 (Fig. 3), plasminogen activator inhibitor-1 (PAI-1), intercellular adhesion molecule-1 (ICAM-1) (Fig. 4), CD4, CD8, CD11c, F4/80, FOXP3 (Fig. 5), p22phox, and p47phox (Fig. 6) relative to SED (all $P < 0.05$). Furthermore, WR resulted in increased expression of PPARGC-1α (Fig. 7), and DR resulted in decreased expression of GRP78 and CHOP (all $P < 0.05$) (Fig. 6). Subcutaneous AT of WR and DR rats exhibited reduced expression of leptin and MCP-1 relative to SED (all $P < 0.05$). Interscapular brown AT of WR and DR rats exhibited reduced expression of leptin (Fig. 3) and PAI-1 (Fig. 4) relative to SED (all $P < 0.05$). Furthermore, WR resulted in increased expression of MCP-1, TNF-α ($P = 0.06$), IL-6 (Fig. 3), E-selectin (Fig. 4), CD4 ($P = 0.06$), F4/80 ($P = 0.06$) (Fig. 5), and p47phox (Fig. 6; all $P < 0.05$ unless otherwise indicated), whereas DR resulted in reduced expression of UCP-1 (Fig. 7; $P < 0.05$). Periaortic AT of WR and DR rats exhibited reduced expression of leptin, MCP-1, TNF-α (Fig. 3), CD11c (Fig. 5), and UCP-1 (Fig. 7) relative to SED (all $P < 0.05$). Furthermore, WR resulted in reduced expression of PAI-1 (Fig. 4), CD8 (Fig. 5), and increased expression of VCAM-1 (Fig. 4; all $P < 0.05$). On the other hand, DR resulted in reduced expression of IL-6 (Fig. 3), E-selectin, ICAM-1 (Fig. 4), CD4, F4/80 (Fig. 5), p22phox, and p47phox (Fig. 6; all $P < 0.05$).

Aorta of WR and DR rats exhibited reduced expression of IL-6 (Fig. 3), E-selectin, VCAM-1 (Fig. 4), CD4, CD8, F4/80 (Fig. 5), GRP78, and CHOP (Fig. 6) relative to SED (all $P < 0.05$). Furthermore, WR resulted in reduced expression of MCP-1 (Fig. 3), CD11c (Fig. 5), and increased expression of PAI-1 (Fig. 4; all $P < 0.05$).

As illustrated in Fig. 8, WR and DR rats exhibited reduced periaortic AT-derived secretion of leptin, IL-6 (all $P < 0.05$), and MCP-1 ($P = 0.09$ and $P = 0.11$, respectively) relative to SED. Periaortic-derived secretion of TNF-α was similar among groups ($P > 0.05$).

Insulin-stimulated aortic relaxation was significantly greater in WR rats relative to SED and DR rats (Fig. 9). Treatment of aortic rings with tezosentan, a nonselective ET-1 receptor blocker, largely removed differences in insulin-stimulated relaxation between WR and SED rats. That is, tezosentan in-
Fig. 4. Expression of plasminogen activator inhibitor-1 (PAI-1) and adhesion molecule-related genes in ATs and aorta of SED, WR, and DR OLETF rats. Values are expressed as means ± SE; n = 10/group. For each gene, SED is used as the reference group and set at 1. *Significant difference (P < 0.05) from SED rats. #Significant difference (P < 0.05) from WR rats.

Fig. 5. Expression of immune cell-related genes in ATs and aorta of SED, WR, and DR OLETF rats. Values are expressed as means ± SE; n = 10/group. For each gene, SED is used as the reference group and set at 1. *Significant difference (P < 0.05) from SED rats. #Significant difference (P < 0.05) from WR rats.
creased insulin-stimulated relaxation in the SED \((P = 0.057)\) but had no effect in WR or DR \((P > 0.05)\). A small but significant \((P < 0.05)\) decrease in ACh-mediated relaxation was observed in DR \((61.6 \pm 1.9\%)\) rats compared with SED \((69.5 \pm 3.1\%)\) and WR \((71.9 \pm 4.5\%)\) rats at the highest dose of ACh. In addition, we observed no differences in ET-1-mediated contraction or SNP-mediated relaxation among groups \((P > 0.05)\).

**DISCUSSION**

The primary findings of the present study are as follows: 1) treatment with voluntary WR and 30% DR regimen for 8 wk (starting at 12 wk of age) resulted in marked reductions in adiposity, improved blood lipid profiles, and systemic markers of insulin resistance in the obese OLETF rat model; 2) reductions in adiposity, through both WR and DR, were associated with decreased AT expression of inflammatory genes and markers of immune cell infiltration, effects that were most pronounced in visceral AT compared with subcutaneous and perivascular AT; 3) in contrast to other depots, markers of inflammation/immune function in brown AT were upregulated with WR but not DR; 4) WR- and DR-induced reductions in expression and secretion of cytokines in perivascular AT were accompanied with a more atheroprotective gene expression profile in the adjacent aortic wall; and 5) WR, but not DR, resulted in increased insulin-stimulated relaxation in the aorta, an effect that was, in part, mediated by a decrease in insulin-induced ET-1-mediated activation/contraction in the aorta of WR.

Existing evidence in animals and humans indicates that exercise results in reduced expression of inflammatory genes and markers of immune cell infiltration in white AT \((9, 18, 28, 30, 60, 61)\). We found that voluntary WR-induced decreases in adiposity produced, by and large, similar downregulation of inflammatory genes in white AT (retroperitoneal) to that produced by decreases in adiposity with 30% DR regimen. Thus, our data support the notion that changes in adiposity appear to be the main driving force for the decreased inflammation in white AT. Further research evaluating the effects of physical activity in the absence of changes in adiposity is needed to elucidate any exercise-specific effects on AT. A unique aspect of the present study was the examination of the effects of WR and DR in several AT depots beyond visceral fat, i.e., subcutaneous, brown, and perivascular AT depots. Overall, our data indicate that the greatest effects of WR and DR on AT gene expression were in visceral AT. While visceral AT is highly susceptible to obesity-mediated inflammation/infiltration of immune cells \((18, 28, 30, 60, 61)\), these data suggest that the phenotype of visceral AT is also highly amenable to reductions in adiposity. Given this close link between visceral AT expansion and inflammation in obesity \((18, 28, 30, 60, 61)\), it is not surprising that measures of central adiposity in humans relate with metabolic and cardiovascular outcomes \((21)\) and that interventions that result in weight loss are associated with improvements in insulin sensitivity and cardiovascular health \((22)\).

An intriguing finding of the present study is that WR and DR produced differential effects on brown AT gene expression.
While both WR and DR produced a downregulation of leptin and PAI-1 mRNA, WR resulted in the induction of proinflammatory genes, markers of immune cell infiltration, and oxidative stress. The finding that WR markedly increased expression of IL-6 in brown AT is of particular interest, considering the recent study by Stanford et al. (54), demonstrating that brown AT-derived IL-6 is required for the profound effects of brown AT transplantation on glucose homeostasis and insulin sensitivity. Indeed, those authors found that the beneficial metabolic effects of brown AT transplantation were lost when AT used for transplantation was obtained from IL-6 knockout mice (54). Our data combined with the results of Stanford et al. (54) suggest that the beneficial metabolic effects of exercise may be, in part, mediated by an increased expression of IL-6 in brown AT. To our knowledge, this is the first study to provide evidence that physical activity, but not calorie restriction, is effective in inducing IL-6 expression in brown AT. Our finding that other proinflammatory markers (e.g., MCP-1, TNF-α) were also upregulated in brown AT warrants further investigation.

Given the increasing evidence implicating AT surrounding large arteries as a local source of inflammatory cytokines that may be involved in the instigation of vascular dysfunction and atherosclerosis (12, 13, 19, 20, 33, 35, 36, 45, 46, 56), a central focus of the present study was to examine the impact of WR and DR on the phenotypic modulation of periaortic AT. Periaortic AT from WR and DR rats clearly exhibited reduced lipid deposition relative to sedentary control rats (Fig. 2), and this effect was associated with reduced markers of immune cell infiltration (CD11c, CD4, CD8). Notably, we also found that WR and DR caused a reduction in periaortic AT-derived secretion of cytokines, such as leptin, IL-6, and MCP-1. To our knowledge, this is the first evidence that physical activity and dietary restriction can effectively reduce expression and secretion of cytokines from perivascular AT. The robust reduction in leptin secretion from periaortic AT induced by WR and DR deserves specific attention, given direct evidence implicating perivascular AT-derived leptin as a potential contributor to vascular dysfunction. In this regard, Payne et al. (45) elegantly demonstrated that perivascular AT-induced impairment of coronary artery function in pigs with metabolic syndrome was reversed with a recombinant leptin antagonist. Furthermore, there is increasing evidence that leptin, when in excess, induces a proinflammatory and prooxidant vascular phenotype (4, 8, 14, 29, 49, 53, 62). Given our findings that WR and DR reduced systemic inflammation and secretion of cytokines from periaortic AT, we hypothesized that these effects would be accompanied by an atheroprotective phenotype in the adjacent aortic wall. Indeed, we found that both WR and DR resulted in downregulation of inflammatory genes (e.g., IL-6, E-selectin, VCAM-1), markers of immune cell infiltration (e.g., CD4, CD8, F4/80), and markers of endoplasmic reticulum stress (e.g., GRP78 and CHOP) in the aorta. These findings are consistent with the hypothesis that at the transcriptional level, the effects of physical activity on vascular cell phenotype may be driven by changes in adiposity and the consequent alterations in cytokine secretion from AT.

In contrast to the results where WR and DR had similar effects on AT and vascular gene expression, our results from the aortic vasomotor function experiments indicate that physical activity improved insulin-induced vasorelaxation, while DR had no effect. This finding is consistent with previous data from our group showing that insulin-stimulated dilation in skeletal muscle arterioles was enhanced in WR, but not DR, OLETF rats (37). Herein, we also report that differences in insulin-stimulated vasorelaxation between WR and SED rats were largely abolished after treatment of aortic rings with a nonselective ET-1 receptor blocker. ET-1 blockade had little to no effect on insulin-induced relaxation in the aorta from WR animals but produced nearly a twofold increase in insulin-induced relaxation in SED. This finding suggests that increases in vasorelaxation to insulin with physical activity were mediated, in part, by a decrease in ET-1 signaling. Furthermore, to evaluate whether greater insulin-stimulated relaxation in the aorta of WR rats was due to reduced vascular sensitivity to ET-1 and/or decreased vascular production of ET-1, we examined aortic responsiveness to exogenous ET-1 in all three groups of animals. We found similar ET-1-mediated constriction among groups, suggesting that it is local insulin-stimulated ET-1 activation and not the vascular sensitivity to ET-1 per se that is likely modulated by physical activity. It should be noted that in the present study, we used tezosentan, a nonselective ET-1 receptor blocker. Future studies should dissect the contribution of ETA vs. ETB receptors using specific blockers.
Our findings that physical activity, but not DR, increases insulin-stimulated vasorelaxation as a result of decreased insulin-mediated ET-1 activation may be significant in light of evidence indicating that excess ET-1 signaling is an important contributor to the pathogenesis of macrovascular disease (47). Exercise-induced increases in blood flow and, thus, shear stress to the artery wall is a likely mechanism by which physical activity exerts an insulin-sensitizing effect on the aorta and a decrease in ET-1 (24, 44). This hypothesis is supported by evidence that 1) shear stress reduces expression of ET-1 in cultured endothelial cells (59), 2) removal of WR for 7 days increases expression of ET-1 in the rat iliac artery (41), 3) rat soleus muscle feed arteries, known to be chronically exposed to high levels of blood flow, display greater insulin-stimulated dilation, as a result of reduced ET-1 activation, than gastrocnemius feed arteries, known to be chronically exposed to lower levels of flow (26), and 4) inactive lower limbs of spinal cord injury patients, chronically exposed to low blood flow and shear stress (3), exhibit an enhanced ET-1-mediated basal vascular tone (58).

Another interesting finding of the present study is that the WR-related increase in insulin-stimulated relaxation in the aorta occurred in the absence of changes in ACh-mediated relaxation, a response largely mediated by nitric oxide in rat aorta. Incidentally, compelling evidence from studies using the Zucker obese rat model indicates that impairments in insulin-mediated dilation occurs prior to impairments in ACh-mediated dilation in both skeletal muscle (15) and coronary arterioles (27, 39). It is possible that ACh-mediated dilation was not improved in our WR animals, relative to the sedentary OLETF

Fig. 8. Secretion of cytokines from periaortic AT explants in SED, WR, and DR OLETF rats. Values are expressed as means ± SE (n = 10/group). *Significant difference (P < 0.05) from SED rats.

Fig. 9. Vasomotor function of thoracic aortic rings in SED, WR, and DR OLETF rats. Values are expressed as means ± SE (n = 10/group). *Significant difference (P < 0.05) from SED rats. #Significant difference (P < 0.05) from WR rats.
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animals, because no impairment in ACh-mediated dilation may have been present in these 20-wk-old sedentary rats. Consistent with this hypothesis, data in animals and humans suggest that exercise training does not further improve endothelium-dependent dilation in subjects with a healthy endothelium, likely due to a ceiling effect (23, 43). We did observe, unexpectedly, that DR resulted in a small but significant decrease in ACh-mediated dilation relative to the SED group fed ad libitum; this effect was not observed in WR animals.

Perspectives and Significance

We provide evidence that reduced adiposity, owing to either increased physical activity or diet restriction, in the obese, insulin-resistant OLETF rat model leads to a marked reduction in the expression of inflammatory genes and markers of immune cell infiltration in visceral and periaortic AT. Our data also demonstrate unique AT depot-specific effects of both increased physical activity and dietary restriction. The anti-inflammatory effects of physical activity and diet restriction on AT were accompanied with a more atheroprotective gene expression profile in the aorta. Importantly, our results indicate that physical activity enhanced aortic insulin-induced relaxation, while diet restriction did not, suggesting that the insulin-sensitizing effect on the vasculature is exercise-specific.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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2. Bailey-Downs LC, Tuscel Z, Toth P, Sosnowska D, Gautham T, Sonntag WE, Csiszar A, Ungvari Z. Exercise training does not further improve endothelium-dependent dilation in subjects with a healthy endothelium, likely due to a ceiling effect (23, 43). We did observe, unexpectedly, that DR resulted in a small but significant decrease in ACh-mediated dilation relative to the SED group fed ad libitum; this effect was not observed in WR animals.


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