Voluntary wheel running improves adipose tissue immunometabolism in ovariectomized low-fit rats

Terese M. Zidon, Young-Min Park, Rebecca J. Welly, Makenzie L. Woodford, Rebecca J. Scroggins, Steven L. Britton, Lauren G. Koch, Frank W. Booth, Jaume Padilla, Jill A. Kanaley, and Victoria J. Vieira-Potter

Department of Nutrition and Exercise Physiology, University of Missouri, Columbia, MO, US; Department of Child Health, University of Missouri, Columbia, MO, US; Department of Molecular and Integrative Physiology, University of Michigan Medical School, Ann Arbor, MI, US; Department of Biomedical Sciences, University of Missouri, Columbia, MO, US; Department of Anesthesiology, University of Michigan Medical School, Ann Arbor, MI, US

ABSTRACT
Loss of ovarian hormones is associated with increased adiposity, white adipose tissue (WAT) inflammation, and insulin resistance (IR). Previous work demonstrated ovariectomized (OVX) rats bred for high aerobic fitness (HCR) are protected against weight gain and IR compared to rats bred for low aerobic fitness (LCR) yet wheel running prevents OVX-induced IR in LCR rats. The purpose of this study was to determine whether adipose tissue immunometabolic characteristics from female HCR and LCR rats differs before or after OVX, and whether wheel running mitigates OVX-induced adipose tissue immunometabolic changes in LCR rats. Female OVX HCR and LCR rats were all fed a high fat diet (HFD) for 11 weeks. Ovary-intact rats (n = 7–8/group) and randomized to either a running wheel or remain sedentary. Following OVX, sedentary LCR rats had greater serum leptin (p < 0.01) and WAT inflammation (p < 0.05) than sedentary HCR. Wheel running normalized the elevated serum leptin and reduced both visceral (p < 0.05) and subcutaneous (p < 0.03) WAT inflammatory markers in the LCR rats. Paradoxically, wheel running increased some markers of WAT inflammation in OVX HCR rats (p < 0.05), which correlated with observed weight gain. Taken together, HCR rats appear to have a healthier WAT immune and metabolic profile compared to LCR, even following OVX. Wheel running improves WAT health in previously sedentary LCR rats. On the other hand, increased WAT inflammation is associated with adiposity gain despite a high volume of wheel running in HCR rats.

Introduction
White adipose tissue (WAT) accumulation has been linked to systemic metabolic dysfunction [1] and insulin resistance (IR) [2,3], increasing susceptibility for cardiovascular disease, type 2 diabetes, and many types of cancer [4]. Menopause (i.e., loss of ovarian hormones) in humans and ovariectomy (OVX) in rodents increases total adiposity and visceral WAT inflammation [5–7]. Recent research indicates that adipocyte dysfunction is an important factor contributing to systemic IR and the related co-morbidities for which postmenopausal women [6,8] and OVX rodents [9] are particularly susceptible.

Rodent models consistently demonstrate that, when maintained under sedentary conditions, OVX is followed by rapid weight gain in the form of WAT [9], which is exaggerated when presented with a high fat diet (HFD) [10]. Previous studies in our laboratory demonstrated that rats selectively bred for high intrinsic aerobic fitness (i.e., high capacity runners, HCR) are protected against the accretion of body fat associated with OVX compared to rats bred for low aerobic fitness (i.e., low capacity runners, LCR) [11]. Despite the OVX-induced increased body weight in the HCR rats fed an HFD, they were still protected from developing systemic IR compared to LCR [10]. The mechanism(s) for this metabolic protection in OVX HCR rats remain unknown. A previous study using proteome profiling [12] found that male HCR rats had a significantly lower WAT inflammatory profile than LCR rats, suggesting HCR rats have healthier WAT. On the other hand, poor WAT immunometabolic ‘health’ may contribute to the development of systemic metabolic dysfunction in LCR rats.

Physical inactivity is also a major driver of WAT inflammation, whereas exercise training has been shown
to reduce visceral WAT inflammation in previously sedentary rodents [13–17]. Similar results have been obtained in humans [18,19]. Whether the effect of exercise-induced reduction of WAT inflammation is independent from exercise-mediated fat loss is unclear, but previous data suggests that the effect is independent of adiposity changes [14]. Likewise, another study [20] has shown that exercise training reduces visceral WAT inflammation from an acute pro-inflammatory insult, supporting a more ‘direct’ anti-inflammatory effect of exercise. Since menopause in humans [21] and OVX in rodents [11,22] reduces physical activity, it is hypothesized that the increased WAT inflammation associated with OVX could be at least partially attributed to physical inactivity. Our previous data showed that increasing physical activity via voluntary wheel running in OVX LCR rats virtually rescued their systemic metabolic profile [23]. Exercise training has also been shown to improve WAT mitochondrial health [24–26], but the mechanism(s) responsible for the systemic metabolic protection conferred by wheel running on OVX-associated metabolic dysfunction in this model have not been well elucidated.

Although most studies on the relationship between exercise and adipose tissue inflammation and how this affects systemic metabolic health have focused on WAT, brown adipose tissue (BAT) has recently emerged as an anti-obesity target [27]. Of note, its activity has been shown in some, but not all, studies to be affected by exercise [25]. Wheel running in young male rats fed an HFD showed significant reductions in BAT adipokines, inflammatory macrophage (F4/80), and T cell (CD8) markers [28]. No previous studies, to our knowledge, have examined the combined effects of OVX plus HFD on BAT inflammation, or the effect of wheel running to mitigate that inflammation.

The goals of the current study were to: 1) compare WAT and BAT immunometabolic characteristics, defined by an inflammatory profile and mitochondrial function, between female LCR and HCR rats across adipose tissue depots (i.e., visceral and subcutaneous WAT and interscapular BAT), 2) determine whether the effects of OVX on WAT and BAT immunometabolic characteristics differ between LCR and HCR rats, and 3) determine whether wheel running can mitigate WAT and BAT dysfunction caused by low fitness and/or OVX. We hypothesized that, across depots, LCR rats would present with greater evidence of WAT and BAT immunometabolic dysfunction compared to HCR rats, whereas voluntary wheel running would improve this profile in both LCR and HCR rats. Further, we sought to determine whether this improved adipose tissue profile associated with improved systemic insulin sensitivity.

Methods

Animals and diet

The HCR/LCR rat model was used to assess the effect of voluntary wheel running (i.e., physical activity) on WAT and BAT inflammation and metabolic dysfunction associated with loss of ovarian hormones. Characterizations of the HCR/LCR rodents have been previously described [11,29–34]. This study is an extension of our previous findings that female LCR rats respond more adversely to OVX than HCR rats in terms of total fat gain, reduced energy expenditure and IR [11], which is exacerbated by an HFD [10]. Randomly cycling female rats aged 25–35 weeks (generation 31–33) were singularly housed under standard temperature (22°C) and humidity on a 12h-12h light/dark cycle. Rats were tested for running capacity at the University of Michigan at 11 weeks of age and shipped to the University of Missouri at 16–20 weeks of age. Animals were initially provided with standard rodent chow (Laboratory Diets 5001) and water ad libitum. Sham (SHM) sedentary (SED) rats (i.e., ovary-intact) remained on standard chow until study completion (N = 7–12 animals/group) [11]. Following OVX, animals were given an HFD (45% kcal fat, D12451, Research Diets) and half were randomly selected to either access running wheels (i.e., voluntary wheel running, VWR) (11 cm wide with an inner diameter of 35 cm; bar running surface) or remain SED (N = 7–8 animals/group) for 11 weeks [23]. National Institutes of Health guidelines were strictly followed and all procedures were approved by the Institutional Animal Care and Use Committee at the University of Missouri prior to study initiation.

Ovariectomy (OVX) and sham (SHM) surgeries

Rat (~28–30 weeks old) OVX or SHM operations were maintained under 2% isoflurane anesthesia and performed as previously described [10,11]. Briefly, a 2.5 cm midline dorsal incision was made followed by two bilateral muscular incisions to expose the ovaries. OVX included removal of the whole ovary, ovarian bursa and part of the oviduct. After ovary removal, the skin incision was closed using wound clips, and acetaminophen (500 mg·kg⁻¹) was administered for post-operative pain management. OVX effectiveness was determined at the conclusion of the study via verification of uterine atrophy, which was observed in each animal from the OVX group. Final uterine weights were 1.03 ± 0.18 g, 0.24 ± 0.02 g, 1.00 ± 0.12 g, and 0.23 ± 0.02 g for SHM HCR, OVX HCR, SHM LCR, and OVX LCR, respectively, with an OVX main effect, p<0.001.

| Animal | Uterine Weight (g) |
|--------|--------------------|
| SHM HCR | 1.03 ± 0.18 |
| OVX HCR | 0.24 ± 0.02 |
| SHM LCR | 1.00 ± 0.12 |
| OVX LCR | 0.23 ± 0.02 |
Assessment of adipose tissue deposition

At sacrifice, rats were assessed for body composition (i.e., total % fat) using dual energy x-ray absorptiometry (DXA) (Hologic QDR-1000, calibrated for rodents) [10,11,23]. Regional fat distribution was further assessed post mortem. The perigonadal, retroperitoneal, omental, and subcutaneous inguinal WAT as well as interscapular BAT depots were weighed to the nearest 0.01g [11]. Body and fat pad weights have been reported previously and are presented herein [10,11,35]. Relative BAT to total visceral WAT ratio was also calculated as BAT (g) / total visceral WAT (g). Total visceral WAT was the sum of the perigonadal, retroperitoneal, and omental depots.

Insulin sensitivity measurements

At six weeks post-OVX, glucose tolerance test (GTT) was used to indirectly assess IR, as previously reported [23]. The Matsuda Index, a measurement of insulin sensitivity, was also calculated using the formula: (10,000 / (fasting glucose × fasting insulin) × mean postprandial glucose × mean postprandial insulin)^0.5 [36]. Insulin and glucose values were presented in pmol·l⁻¹ and mmol·l⁻¹, respectively. At 11 weeks post-OVX, five hours prior to sacrifice, at the beginning of the light cycle (6:00am), running wheels were removed to reduce any exercise effects, fasting blood samples were taken, and surrogate measures of IR were calculated via the homeostatic model assessment of IR (HOMA-IR) (fasting insulin (uIU/mL) × fasting glucose (mmol/L))/22.5 and AT IR (Adipo-IR) fasting insulin (pg/mL) × fasting NEFA (mmol/L)^23 and are presented here.

Fluorescence-associated cell sorting analysis

The stromal vascular cell (SVC) fraction was isolated from extracted visceral WAT (from the perigonadal WAT depot only) via mincing followed by collagenase digestion as previously described [7]. SVCs were then diluted to 0.25 × 10⁶ cells/100 uL in cold (4°C) sorting buffer (PBS containing 1 mmol/liter EDTA, 25 mmol/liter HEPES, and 1% fatty acid free BSA) until immunolabeling. The following rat-specific fluorochrome-conjugated antibodies were used: CD3, CD4, and CD11c (BD Biosciences: Catalog #561801, 561579, and 562222). The fluorochrome conjugated antibodies allowed us to quantify the total percentage of SVCs that were CD3+ (i.e., total T lymphocytes), the percentage of CD3+T cells that were CD4+CD8- (i.e., effector/memory T lymphocytes) and the percentage of CD11c (i.e., M1/inflammatory macrophages). Gating strategies included dead cell discrimination and lymphocyte quantification based on forward/side scatter and included unstained cells, single stain positive controls, and FMO controls. Cells were immunophenotyped using a CyAn ADP Analyzer (Beckman Coulter, Inc.) and data analyzed using FlowJo (FlowJo, LLC).

RNA extraction and real-time PCR

Whole perigonadal and subcutaneous WAT, and interscapular BAT samples were homogenized in a Qiazol solution (Qiagen Catalog #79306) using a tissue homogenizer (TissueLyser LT, Qiagen). Total RNA was isolated using the Qiagen’s RNeasy Lipid Tissue Kit and assayed using a Nanodrop spectrophotometer (Thermo Scientific) to assess purity and concentration. First-strand cDNA was synthesized from total RNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems). Quantitative real-time PCR was performed as previously described [37] using the ABI StepOne Plus sequence detection system (Applied Biosystems). Primer sequences (Table 1) were designed using the NCBI Primer Design tool. All primers were purchased from IDT (Coralville, IA). A 20-μl reaction mixture containing 10 μl iTaq Universal SYBR Green SMX (BioRad, Hercules, CA) and the appropriate concentrations of genespecific primers plus 4 μl of cDNA template were loaded in each well of a 96-well plate. All PCR reactions were performed in duplicate. PCR was conducted with thermal conditions as follows: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 45 s. A dissociation melt curve analysis was performed to verify the specificity of the PCR products. 18S primers were used to amplify the endogenous control product (i.e., HK gene). mRNA expression values are presented as 2ΔΔCT whereby ΔΔCT = HK CT – gene of interest CT; data are expressed as fold difference relative to the respective control group (HCR ovary-intact or HCR OVX sedentary).

| Table 1. PCR primer sequences. |
|-----------------------------|
| **CD3** | ATGAGCTGCAAGGCTGCTTCA | ATGGTCCCCAGAAGAGGCTTCA |
| **CD8** | CACAGGCTCCACCGTCCCTG | GCGAGCTCCGAGCTTCA |
| **CD11c** | CTGCTATCGACAGCCAGCA | AGTGGTCCCCGAGCTTCA |
| **CD4** | ACCCTAAGGCTCTGACCCC | TAGGGTCCCCGAGCTTCA |
| **TNFα** | CTGGGGGAGCTGTGAGGA | CCCAGAAGAGGCTCAGCAG |
| **IL-6** | AGTCATCCCCGAGCTTCA | GCGAGCTCCGAGCTTCA |
| **F4/80** | ATAGGCGAAGGCTCTGACCCC | GCGAGCTCCGAGCTTCA |
| **MCP1** | CACAGGCTCCACCGTCCCTG | GCGAGCTCCGAGCTTCA |
| **Adiponectin** | CAAGGGCTTCCCTC | CCAGGACCCTCAGGCA |
| **Leptin** | CAGGCATCGAGGCTGCTTCA | ACCAAGGCTCGCTGAT |
| **18s** | GCCGCTAGAGGCTGCTTCA | CATTCTGCGAAGGCTTCA |
Serum adiponectin and leptin

Serum adiponectin (μg/mL) and leptin (pg/mL) were measured using ELISA commercially available kits (Crystal Chem; #80750 and #90040) and completed according to the manufacturer’s protocol. The adiponectin/leptin ratio was calculated adiponectin (pg/mL)/leptin (pg/mL)/1000, indicative of IR [38,39].

Western blot analysis

Western blotting was performed as previously described [[40], [41]]. Triton X-100 cell lysates were used to produce Western blot-ready Laemmli samples from the retroperitoneal WAT and BAT. Samples were separated by SDS-PAGE, transferred to polyvinylidene difluoride membranes, and probed with primary antibodies. Antibodies against oxidative phosphorylation (OxPhos) complexes was purchased from Abcam (ab110413; Abcam, Cambridge, MA). Individual protein bands intensity were quantified using FluoroChem HD2 (AlphaView, version 3.4.0.0), and expressed as a ratio to the gel standard. To control for equal protein loading and transfer, the membranes were then stained with 1% amido-black (Sigma). The total protein staining for each lane was quantified and these values were used to correct for any differences in protein loading or transfer of all band densities [41].

Statistical analysis

All data were analyzed using SPSS v23.0 (IBM statistics software). Student’s t-tests were used to determine line (i.e., HCR vs LCR) differences between ovary-intact animals. Two-way analysis of variance (ANOVA) was used to determine differences for line main effect (HCR vs LCR) and treatment (SED vs. VWR) and line by treatment interaction for all OVX rodents. Post hoc LSD tests were performed when appropriate. Bivariate Pearson’s correlations were performed to determine associations following OVX. All data are expressed as mean ± SEM and significance was at p < 0.05.

Results

Body composition and insulin resistance

Analyses of body composition and IR have been previously reported [11, 23], but those data are summarized in Figure 1 and Table 2. Ovary-intact HCR and LCR rats gained weight during the 11 week HFD-feeding study period (+4.5% in HCR and +6.4% in LCR; Fig. 1A) and LCR had higher final body weights (p<0.001; Table 2) and adiposity at the end of the study (p<0.001; Fig. 1B), OVX significantly increased body weight in both HCR and LCR rats (+28.7% in HCR and +32.0% in LCR; p<0.001 compared to ovary-intact HCR and LCR, respectively) (Fig. 1A). Those increases paralleled increases in adiposity (p<0.001; Fig. 1B) [11, 23]. OVX HCR rats with access to a running wheel ran 5.5 ± 2.0 km/day and OVX LCR ran 1.3 ± 0.8 km/day [23]. Wheel running attenuated weight gain in the OVX LCR (−11.1%), yet increased body weight in HCR rats (+14.6%) (line × treatment interaction, p<0.001; Fig. 1A-B), despite increased energy expenditure (Table 2). The increased weight gain via adiposity was likely due to HFD overconsumption with wheel running in the HCR rats. Food intake (45% kcal/g from fat) for OVX-HCR SED: 12.70 ± 0.40 g/day vs VWR: 16.84 ± 0.77 g/day (+33%) and for OVX-LCR SED: 14.16 ± 0.37 g/day vs VWR: 13.13 ± 0.63 g/day; a two-way ANOVA was conducted and there was a significant line × treatment interaction (p<0.001), as previously reported [23]. Those divergent trends were consistent with changes in subcutaneous and visceral WAT depot weights (line × treatment interaction, p<0.05; Fig. 1C-D). Although no differences were detected in BAT (Fig. 1E), the relative amount of BAT (i.e., BAT to total visceral WAT ratio) was lower in the ovary-intact LCR compared to HCR rats (p<0.05; Fig. 1F). Following OVX, this ratio was reduced but increased with wheel running in both groups (p = 0.05).

Voluntary wheel running significantly attenuated OVX-induced systemic IR, measured via HOMA-IR and Adipo-IR in OVX LCR rats (p<0.05; Fig. 1G-H). In contrast, wheel running had the opposite effect in OVX HCR rats (both line × treatment interaction, p<0.04). A Student’s t-test between the OVX-HCR VWR and OVX-LCR VWR IR revealed no significant difference between the two groups (p = 0.6).

Adipose tissue depot-specific immunological changes

Visceral WAT

A greater percentage of adipose tissue stromal vascular cell (SVC) fraction consisted of CD3+ (non-specific T cell surface marker), CD4+ (T helper cell surface marker) T cells, and CD11c+ (inflammatory) macrophages in the ovary-intact LCR compared to HCR rats (Fig. 2A-C). Similarly, ovary-intact LCR rats had greater CD4 (p<0.05; Fig. 2E) and tumor necrosis factor alpha (TNFα) (pro-inflammatory cytokine secreted by macrophages; trend p = 0.08; Fig. 2H) gene expression compared to HCR rats in that same depot. Following OVX, LCR rats maintained significantly greater TNFα and monocyte chemoattractant protein 1 (MCP1), (line
effect, p<0.05) (Fig. 2H, K) gene expression compared to HCR with no differences for the remaining inflammatory gene expressions between LCR and HCR rats following OVX. In LCR rats following wheel running, reductions were evident in inflammatory T cell infiltration CD3+ SVC T cells (line × treatment interaction, p = 0.04; Fig. 2A), CD4 (line × treatment interaction, p = 0.04; Fig. 2E), and F4/80 (non-specific macrophage marker) (line × treatment interaction, p = 0.03; Fig. 2J) gene expression. The significant interactions indicate that, while wheel running reduced inflammation in WAT of LCR rats, it had the opposite effect in HCR rats. This paralleled the increased adiposity in the OVX HCR wheel runners. Other inflammatory genes were not different following wheel running (Fig. 2B-D, F-I, K).
Subcutaneous WAT

Ovary-intact LCR had greater CD8 (cytotoxic T cell marker, trend p = 0.08; Fig. 3D) and lower F4/80 (p = 0.002; Fig. 3G) gene expression compared to ovary-intact HCR rats with no other differences in inflammatory markers. CD8 gene expression remained greater...
following OVX in LCR compared to HCR rats (main line effect, \( p = 0.05 \); Fig. 3D), but no change with wheel running. In OVX LCR rats, voluntary wheel running reduced gene expression of the macrophage markers CD11c (treatment main effect, \( p = 0.04 \); Fig. 3C) and F4/80 (line \( \times \) treatment interaction, \( p = 0.02 \); Fig. 3G). No other inflammatory genes were altered by wheel running (Fig. 3A-B, E-F, H).

**Brown adipose tissue (BAT)**

BAT gene expression of CD11c was significantly lower in ovary-intact LCR compared to HCR rats (\( p<0.05 \); Fig. 4C). Neither intrinsic fitness nor wheel running had a significant effect on indices of BAT inflammation following OVX (Fig. 4A-H). Wheel running tended to reduce TNF\( \alpha \) and IL-6 expression in HCR while they trended toward being increased in LCR (line \( \times \) treatment interaction, \( p = 0.06 \) and \( p = 0.08 \), respectively; Fig. 4E-F).

**Adiponectin and leptin levels in OVX animals**

No differences were detected in circulating adiponectin levels between ovary-intact HCR and LCR animals. Following OVX, there was a trend toward higher adiponectin levels in the LCR compared to SED HCR, but circulating adiponectin was not affected by wheel running in either line (\( p = 0.07 \); Fig. 5A). There were also no adiponectin gene expression differences in either visceral or subcutaneous WAT (Fig. 5D-E). Following OVX, BAT adiponectin gene expression was greater in the LCR compared to HCR rats (line main effect, \( p = 0.02 \); Fig. 5F); again, there was no wheel running effect.

Circulating leptin concentrations were not different between ovary-intact HCR and LCR rats (Fig. 5B). However, following OVX, an increase in circulating leptin was evident in LCR rats (line main effect, \( p = 0.04 \)), which was completely normalized by wheel running (treatment main effects, \( p = 0.02 \); Fig. 5B); this reduction correlated with reductions in leptin gene expression in all LCR fat.
depots (Fig. 5G-I; Table 3). On the other hand, increases in leptin expression were evident following wheel running in OVX HCR animals (line × treatment interaction, p = 0.03; Fig. 5G). This correlated with increased leptin expression in the subcutaneous WAT in the OVX LCR rats (line × treatment interaction, p < 0.01; Fig. 5H). Reductions in leptin expression were detected in BAT following wheel running (treatment p < 0.05; Fig. 5I). Across groups, elevated serum leptin correlated positively with leptin gene expression in the visceral (r = 0.60, p < 0.001) and subcutaneous WAT (r = 0.79, p < 0.001; Table 3). Notably, the OVX HCR wheel runners had 2-fold higher leptin gene expression in the visceral WAT compared to sedentary animals, which significantly correlated with increased visceral mass (r = 0.54, p < 0.05).

The adiponectin to leptin ratio has been shown to be inversely related to BMI in women with polycystic ovary syndrome [38] and is used as an index of insulin sensitivity [39]. In the present study, the adiponectin to leptin ratio was not significantly different between ovary-intact HCR and LCR animals nor were there differences detected following OVX or wheel running in these animals (Fig. 5C). However, the adiponectin to leptin ratio correlated inversely with both HOMA-IR and Adipo-IR (r = 0.59, p < 0.01; Table 3).

**Adipose tissue mitochondrial oxidative phosphorylation protein analyses**

In comparing ovary-intact LCR and HCR rats, visceral WAT complex II protein content was lower in LCR (p = 0.01; Fig. 6C); whereas, there were no detectable differences in BAT mitochondrial complex content (Fig. 6E). Following OVX, wheel running tended to reduce complex III protein content in the visceral WAT of the LCR and increase it in HCR rats (line × treatment interaction, p = 0.06; Fig. 6D). In the BAT, there was evidence of higher mitochondrial complex II and III content (both line × treatment interactions, p < 0.05; Fig. 6F) following OVX in LCR, which was normalized with wheel running; wheel running increased these complexes in the HCR animals.
Correlations for OVX HCR and LCR cohort

Correlations between metabolic analyses (e.g., serum leptin), adiposity, and IR were assessed (Table 4). Notably, although serum adiponectin was not significantly correlated with adiposity or IR, there was a strong negative association between the adiponectin to leptin ratio and adiposity. Several genes and mitochondrial complexes from various AT depots were found to be positively correlated with adiposity (i.e., visceral WAT and total fat percentage) and/or IR (i.e., HOMA-IR and Adipo-IR) and are presented in Table 4.

Discussion

Menopausal women and OVX rodents have a high propensity for developing systemic and WAT inflammation [6,7,13,42] with corresponding obesity-induced systemic IR. They also experience a significant reduction in physical activity [11,21], which is associated with increased prevalence of 40 chronic conditions/diseases [43]; whereas exercise training improves systemic IR and reduces WAT inflammation [14–16,44]. Chronically, reduced physical activity leads to reduced aerobic fitness, whereas low aerobic fitness itself adversely affects systemic metabolism [11,33] and may induce AT dysfunction [11,12,45]. Herein, we tested whether wheel running restores systemic metabolic health by improving AT “immunometabolism” following OVX in low-fit (e.g., LCR) rodents. The novel findings are: 1) Ovary-intact LCR female rats on low-fat chow diet had greater visceral WAT inflammation and leptin levels compared to HCR, 2) voluntary wheel running reduced specific key inflammatory markers in the visceral and subcutaneous WAT and normalized leptin levels following OVX in the LCR rats, 3) contrary to our hypotheses, wheel running increased adiposity and some indicators of WAT inflammation in the OVX HCR rats, but did not significantly impact WAT or BAT mitochondrial measures in either line.

Increased WAT inflammation contributes to systemic metabolic dysfunction [46]. The present findings
demonstrated that ovary-intact, sedentary LCR rats had greater evidence of visceral WAT immune cell infiltration, indicated by elevated CD3+ and CD4+ (T cell) and CD11c+ (pro-inflammatory macrophage) SVCs, compared to ovary-intact HCR. Only two previously published reports have measured WAT-specific changes in immune and metabolic indicators in this rodent model and both were performed in male rats [12,45]. One study compared the visceral WAT-specific mitochondrial functional differences between HCR and LCR rats fed a standard chow diet and found that, although citrate

| Table 3. Pearson correlations for leptin in OVX LCR and HCR with and without running wheel. |
|-----------------------------------------------|
| Serum leptin (ng/mL) | Corresponding fat depot |
| Visceral WAT leptin mRNA | 0.60**  | 0.53** |
| Subcutaneous WAT leptin mRNA | 0.79***  | 0.69*** |
| BAT leptin mRNA | 0.44*  | 0.63** |

***Correlation is significant when \( p < 0.001 \)

**Correlation is significant when \( p < 0.01 \)

*Correlation is significant when \( p < 0.05 \)

Figure 6. Mitochondrial expression analyses across adipose tissue depots in HCR vs. LCR rats. Mitochondrial oxidative phosphorylation complexes I, II, III, IV, and V for (A-B) retroperitoneal WAT (VAT) and (D-E) BAT. Representative western blot images for (C) VAT and (F) BAT. *Indicates significant differences between ovary-intact (SHM) rats. Values are mean ± SEM; where \( p < 0.05 \) is statistically significant. L = line main effect, HCR vs. LCR; T = treatment main effect, SED vs. VWR; LxT = line by treatment interaction. BAT = brown AT; HFD = high fat diet; NC = standard normal chow; SED = sedentary; VWR = voluntary wheel running.
The current study revealed higher visceral WAT mitochondrial enzyme changes and biogenesis, citrate synthase activity, and proteins involved in oxidative phosphorylation in the visceral WAT following a swimming exercise regimen, which is known to be a vigorous and perhaps stressful intervention. Another study [52] using male C57Bl/6 mice found robust increases in WAT mitochondrial biogenesis, citrate synthase activity, and cytochrome c oxidase subunit IV following 10 weeks of wheel running. Although the present study solely examined mitochondrial oxidative phosphorylation enzyme changes, minimal changes were observed following wheel running (i.e., complex III, Table 3). In light of the above evidence from other studies that exercise enhances adipose tissue mitochondrial adaptations, the reason for the lack of exercise-induced mitochondrial effects in visceral WAT in the current study is unclear. However, it is important to note that the current study was performed in female rats, whereas those other

**Table 4. Correlations for OVX LCR and HCR with and without running wheel.**

|                       | Visceral fat (g) | Total fat % | HOMA-IR | Adipo-IR |
|-----------------------|-----------------|-------------|---------|----------|
| HOMA-IR               | 0.65**          | 0.54**      | 0.87**  |          |
| Adipo-IR              | 0.66**          | 0.60**      | 0.87**  |          |
| Serum adiponectin      | 0.34            | 0.38        | 0.06    | 0.11     |
| Serum leptin           | 0.79**          | 0.89**      | 0.61**  | 0.67**   |
| Adiponectin/leptin ratio| -0.75**        | -0.76**     | -0.59** | -0.59**  |

**Correlation is significant when p < 0.001**
**Correlation is significant when p < 0.01**
*Correlation is significant when p < 0.05

compared to the HCR rats. However, whether this was due to their greater adiposity or reduced physical activity (or both) is not clear.

Exercise is well recognized as an intervention to prevent fat accumulation and improve WAT inflammation [14,15,50] by reducing pro-inflammatory cytokine expression [16,50] independent of a change in fat mass [14]. We found that a low volume of wheel running in the LCR reduced total fat mass (−52%) and reduced inflammatory markers in the visceral (e.g., CD3+ T cells and T cell and macrophage-related mRNA) and subcutaneous (e.g., macrophage mRNA) WAT. Whether the improved inflammatory profile is solely due to either the increased physical activity or the attenuated fat mass cannot be definitively established in this study, yet increased adiposity is clearly associated with WAT inflammation (Table 3), as indicated by numerous other studies. However, a unique finding in the present study was that wheel running in the OVX HCR rats caused weight gain as well as a greater inflammatory WAT profile. This may suggest that inflammatory effects of HFD-induced adipose expansion outweigh the protective anti-inflammatory effects of exercise. That is, the gain in adiposity in the HCR rats may have prevented the anti-inflammatory effects of exercise in WAT. Nonetheless, whether HFD-fed adiposity-matched OVX HCR rats kept sedentary would have greater inflammation than those studied here, who performed high volumes of wheel running, is not known but is likely the case. Clearly, further studies are needed to answer these questions. Future studies should include a weight-matched OVX HCR group kept sedentary to tease out the effect of exercise on adipose tissue parameters.

Exercise has also been shown to increase mitochondrial enzymes in WAT [51,52]. A study [51] examining young male rats found increased mitochondrial biogenesis, citrate synthase activity, and proteins involved in oxidative phosphorylation in the visceral WAT following a swimming exercise regimen, which is known to be a vigorous and perhaps stressful intervention. Another study [52] using male C57Bl/6 mice found robust increases in WAT mitochondrial biogenesis, citrate synthase activity, and cytochrome c oxidase subunit IV following 10 weeks of wheel running. Although the present study solely examined mitochondrial oxidative phosphorylation enzyme changes, minimal changes were observed following wheel running (i.e., complex III, Table 3). In light of the above evidence from other studies that exercise enhances adipose tissue mitochondrial adaptations, the reason for the lack of exercise-induced mitochondrial effects in visceral WAT in the current study is unclear. However, it is important to note that the current study was performed in female rats, whereas those other

synthase activity was ~50% higher in HCR compared to LCR, no other mitochondrial indicators differed between groups [45]. AT immune cell markers were not reported. Another study profiled 448 proteins, 50% of which were associated with mitochondrial function, in the visceral WAT of HCR and LCR rats and found no differences in mitochondrial protein abundance [12]. Furthermore, compared to HCR, LCR rats had greater inflammatory expression (particularly, increased B cell activity) [12]. Similarly, we found higher WAT inflammation in both the ovary-intact and OVX female LCR rats compared to HCR. It is worth noting that male rodents are more susceptible to WAT inflammation compared to females [47], who have been shown to be somewhat protected from WAT inflammation and systemic IR unless ovarian hormone production is inhibited [7, 48].

The mechanism(s) by which OVX increases WAT inflammation are not fully understood. Because some evidence from animal studies indicates that estrogen replacement rescues the dysfunctional WAT phenotype associated with OVX and restores systemic insulin sensitivity [49], this would implicate estrogen loss as a potential mechanism. Whether OVX-mediated WAT inflammation is due to the overall adiposity increase that also occurs is not clear, but previous work demonstrated that OVX increases inflammatory cell influx and inflammation in the visceral WAT independent of total adiposity changes [7]. Evidence in mice suggests that OVX-induced increases in immune cell infiltration and activation in visceral WAT predicts their systemic metabolic impairments [7,9]. The current study revealed higher levels of selected visceral and subcutaneous WAT inflammation following OVX in the sedentary LCR
studies were conducted in male rodents. Female rodents are considerably more active than males, and estrogen may facilitate mitochondrial adaptations in adipose tissue [53]. Unfortunately, few studies have investigated the effects of exercise on adipose tissue mitochondrial function in female rodents. It is also noteworthy that adiposity gain is also associated with changes in mitochondrial markers; thus, the lack of exercise effect in the HCR may have been due to the concomitant increase in adiposity. The lack of effect even in the LCR, who did not experience gains in adiposity, may be due to the much lower dose of exercise performed in that group. That is, a critical threshold of exercise may be necessary to induce significant mitochondrial changes in adipose tissue. Importantly, we did not directly measure mitochondrial enzyme activity or respiration in the adipose tissue; this is a limitation to be addressed in future studies.

It is well established that females are more insulin sensitive than males and that this protection is lost following menopause [54] and OVX in rodents [55]. Our lab has previously shown that HCR rats are more insulin sensitive than LCR rats [11] and are partially protected from OVX-induced IR [10,55]. Paradoxically, wheel running increased adiposity following OVX in HCR rats, despite the five-fold higher volume of running (5.5 km/day) compared to LCR (1.3 km/day) who experienced an attenuation of weight gain with wheel running [23]. Notably, OVX in the HCR rats reduced their volume of running by ~70% compared to the ovary-intact condition (~17.0 km/day) [35,56]. The reduced running volume, combined with increased energy consumption via HFD [23], likely contributed to the increased weight gain following OVX. Surprisingly, despite weight gain and HFD consumption, these rats maintained a relatively healthy systemic metabolic profile, at least at six weeks post-OVX, compared to their sedentary counterparts. Metabolic profile was measured by reduced fasting blood glucose and improved insulin sensitivity, calculated via the Matsuda Index, as previously reported [23]. However, by 11 weeks post-OVX, a significant interaction was evident, where HOMA-IR and Adipo-IR were differentially affected by wheel running (Fig 1). That is, running exacerbated those values in HCR and improved them in LCR, despite the fact that LCR performed only a low volume of running [35]. Those metabolic discrepancies regarding insulin sensitivity/resistance may be due, in part, to an acute exercise effect during the glucose tolerance measurements since the wheels were taken away just two hours prior to GTT testing at the 6 week time point, whereas in the current study analyses at 11 weeks post-OVX, the wheels were removed five hours prior to taking fasting samples at sacrifice. As was the case with the HCR rats, LCR also experienced and OVX-induced reduction in the volume of running compared to the ovary-intact condition. They ran ~11.7 km/day [35,56] versus ~1.0 km/day (a 92% reduction) following OVX. Yet, they were protected from developing IR compared to the LCR sedentary rats [23].

Adiponectin is an adipose-tissue derived anti-inflammatory adipokine, which has been associated with improved insulin action, has also been shown to be down-regulated in obesity [57]. Similar to previous research using a standard chow diet in male HCR and LCR rats [30] we found no differences in serum adiponectin concentrations between the ovary-intact female LCR rats compared to HCR, nor any detectable gene expression differences. Likewise we also found no serum adiponectin differences following OVX in HCR and LCR rats or with wheel running. However, serum leptin concentrations were 6-fold higher in the OVX LCR sedentary rats, whereas wheel running normalized leptin levels. Reduction with running coincided with reduced visceral and subcutaneous WAT and BAT leptin mRNA in the OVX LCR rats. This is not surprising given that high circulating leptin associates with increased adiposity [58] and adipose tissue inflammation [59,60] both of which were observed in the OVX LCR sedentary rats. Although, a similar study in Wistar rats fed an HFD following OVX found reduced circulating leptin levels after six weeks of exercise treadmill training [61] even in the absence of weight loss; thus, exercise itself may reduce leptin levels, which may be attributed to enhance leptin sensitivity. Interestingly, in the OVX HCR rats with a running wheel, although we found a significant increase in leptin mRNA expression in the visceral WAT, we did not see marked increase in the subcutaneous WAT or BAT, or in circulation. Since those animals also gained significant adiposity, this supports the hypothesis that exercise may buffer obesity-induced leptin resistance.

This study offered a rare opportunity to compare the immunometabolic features across fat depots in female rats differing in ovarian status, fitness level, and access to wheel running, in order to better understand the roles of intrinsic fitness, physical activity, and ovarian hormone loss on fat immunometabolism. In addition, it offered the unique and surprising opportunity to investigate the combined effects of weight gain and high level of physical activity. This is the first time, to the best knowledge of the authors, a high volume of exercise coincided with adiposity gain; thus, this finding may offer important insight into the effects of exercise on adipocyte health, including leptin sensitivity. Finally, no previous studies have investigated how intrinsic aerobic fitness may offer protection against OVX-associated adipose tissue inflammation.

The current study is not without limitations. Much of the gene expression analyses was trending toward
significance, but did not reach it. This may be that the number of animals in each group (n = 7–8) did not provide sufficient statistical power which should be considered for future studies. It is also limiting that histological analyses of macrophage infiltration were not assessed, nor were adipocyte cell size analyses performed; these are measures that certainly warrant further investigation. Further, more direct assessments of mitochondrial energetics need to be performed in future studies; and, along those lines, similar studies should be performed at thermoneutrality since temperature is now known to affect adipocyte immunometabolism. Finally, direct comparisons between the ovary-intact and OVX HCR and LCR rats regarding crosstalks between the ovary-intact and OVX HCR and LCR adipocyte immunomnetabolism. Finally, direct comparisons between the ovary-intact and OVX HCR and LCR rats regarding flow cytometry and gene expression analyses were not possible because tissue from those two cohorts were taken at different times.

In summary, these data reveal that female LCR sedentary rats, either ovary-intact or following OVX, have greater WAT inflammation than HCR. Thus, HCR animals are somewhat protected against developing WAT inflammation. Interestingly, in the OVX LCR rats, a low volume of wheel running reduced WAT inflammation and serum leptin levels, suggesting that even a low amount of physical activity is adequate for improvements of adipose tissue health in LCR rats. In contrast, despite a much higher volume of wheel running compared to LCR, the OVX HCR rats developed increased adiposity and WAT inflammation compared to sedentary controls, suggesting that exercise is not effective in protecting against WAT inflammation in the environment of adiposity gain in the current experiment. Reduced fat mass may be required for exercise-induced anti-inflammatory effects to manifest in WAT. Since the weight gain in HCR following OVX under the wheel running condition resulted in increased surrogate indicators of IR by the end of the study, this suggests that adipose tissue expansion is a major driver of IR, and that voluntary running distances in the current experiment were not sufficient to prevent adiposity-induced IR. In conclusion, in previously sedentary LCR rats, a low volume of voluntary wheel running protects against the development of OVX induced disturbances in adipose tissue immunometabolism and systemic IR. However, the parallel studies in OVX HCR rats demonstrated that voluntary exercise, even when performed at high volumes, is not sufficient to protect against the AT inflammation and IR that likely occurs due to excess adiposity under the condition of OVX.

Supported by

This work was supported by a MU Research Council grant (VVP), NIH grant P40OD021331 (LGK and SLB), and NIH grant RO1DK088940 (JP).

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgments

This work was supported by a MU Research Council grant (VVP) and an NIH grant K01 HL-125503 (JP). The LCR-HCR rat model system was funded by the Office of Research Infrastructure Programs grant P40OD021331 (LGK and SLB) from the National Institutes of Health. We acknowledge the expert care of the rat colony provided by Chauna Black. Contact LGK (lgkoch@umich.edu) or SLB (brittons@umich.edu) for information on the LCR and HCR rats: these rat models are maintained as an international resource with support from the Department of Anesthesiology at the University of Michigan, Ann Arbor, Michigan.

Funding

MU Research Council Grant, NIH grant, K01 HL-125503, NIH grant, P40OD021331

References

[1] Despres JP. Is visceral obesity the cause of the metabolic syndrome? Annals of medicine. 2006;38:52–63.
[2] Gabriely I, Ma XH, Yang XM, et al. Removal of visceral fat prevents insulin resistance and glucose intolerance of aging: an adipokine-mediated process? Diabetes. 2002;51:2951–8.
[3] Tchernof A, Despres JP. Pathophysiology of human visceral obesity: an update. Physiol Rev. 2013;93:359–404.
[4] Doyle SL, Donohoe CL, Lysaght J, et al. Visceral obesity, metabolic syndrome, insulin resistance and cancer. Proc Nutr Soc. 2012;71:181–9.
[5] Pfieilschifer J, Koditz R, Pfohl M, et al. Changes in proinflammatory cytokine activity after menopause. Endocr Rev. 2002;23:90–119.
[6] Lee CG, Carr MC, Murdoch SJ, et al. Adipokines, inflammation, and visceral adiposity across the menopausal transition: a prospective study. J Clin Endocrinol Metab. 2009;94:1104–10.
[7] Vieira Potter VJ, Strissel KJ, Xie C, et al. Adipose tissue inflammation and reduced insulin sensitivity in ovarietomized mice occurs in the absence of increased adiposity. Endocrinology. 2012;153:4266–77.
[8] Stefanska A, Bergmann K, Sypniewska G. Metabolic Syndrome and Menopause: Pathophysiology, Clinical and Diagnostic Significance. Adv Clin Chem. 2015;72:1–75.
[9] Rogers NH, Perfield JW, 2nd, Strissel KJ, et al. Reduced energy expenditure and increased inflammation are early events in the development of ovarietomy-induced obesity. Endocrinology. 2009;150:2161–8.
[10] Park YM, Kanaley JA, Zidon TM, et al. Ovariectomized Highly Fit Rats Are Protected against Diet-Induced Insulin Resistance. Med Sci Sports Exerc. 2016;48:1259–69.
[11] Vieira-Potter VJ, Padilla J, Park YM, et al. Female rats selectively bred for high intrinsic aerobic fitness are
protected from ovariectomy-associated metabolic dysfunction. Am J Physiol Regul Integr Comp Physiol. 2015;308:R530–42.

[12] Bowden-Davies K, Connolly J, Burghardt P, et al. Label-free profiling of white adipose tissue of rats exhibiting high or low levels of intrinsic exercise capacity. Proteomics. 2015;15:2342–9.

[13] Baynard T, Vieira-Potter VJ, Valentine RJ, et al. Exercise training effects on inflammatory gene expression in white adipose tissue of young mice. Mediators of inflammation. 2012;2012:767953.

[14] Vieira VJ, Valentine RJ, Wilund KR, et al. Effects of exercise and low-fat diet on adipose tissue inflammation and metabolic complications in obese mice. Am J Physiol Endocrinol Metab. 2009;296:E1164–71.

[15] Kawanishi N, Mizokami T, Yano H, et al. Exercise attenuates M1 macrophages and CD8+ T cells in the adipose tissue of obese mice. Med Sci Sports Exerc. 2013;45:1684–93.

[16] Kawanishi N, Yano H, Yokogawa Y, et al. Exercise training inhibits inflammation in adipose tissue via both suppression of macrophage infiltration and acceleration of phenotypic switching from M1 to M2 macrophages in high-fat-diet-induced obese mice. Exercise immunology review. 2010;16:105–18.

[17] Crissey JM, Jenkins NT, Lansford KA, et al. 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. 2014;306:R596–606.

[18] Bruun JM, Helge JW, Richelsen B, et al. Diet and exercise reduce low-grade inflammation and macrophage infiltration in adipose tissue but not in skeletal muscle in severely obese subjects. Am J Physiol Endocrinol Metab. 2006;290:E961–7.

[19] Kadoglu NP, Perrea D, Iliadis F, et al. Exercise reduces resistin and inflammatory cytokines in patients with type 2 diabetes. Diabetes Care. 2007;30:719–21.

[20] Castellani I, Root-Mccaig J, Frenodo-Cumbo S, et al. Exercise training protects against an acute inflammatory insult in mouse epididymal adipose tissue. J Appl Physiol (1985). 2014;116:1272–80.

[21] Duval K, Prud’homme D, Rabasa-Lhoret R, et al. Effects of the menopausal transition on dietary intake and appetite: a MONET Group Study. Eur J Clin Nutr. 2014;68:271–6.

[22] Izumo N, Ishibashi Y, Ohba M, et al. Decreased voluntary activity and amygdala levels of serotonin and dopamine in ovariectomized rats. Behavioural brain research. 2012;227:1–6.

[23] Park YM, Padilla J, Kanaley JA, et al. Voluntary Running Attenuates Metabolic Dysfunction in Ovariectomized Low-Fit Rats. Med Sci Sports Exerc. 2017;49:254–64.

[24] Xu X, Ying Z, Cai M, et al. Exercise ameliorates high-fat diet-induced metabolic and vascular dysfunction, and increases adipocyte progenitor cell population in brown adipose tissue. Am J Physiol Regul Integr Comp Physiol. 2011;300:R1115–25.

[25] Stanford KI, Middelbeek RJ, Goodyear LJ. Exercise Effects on White Adipose Tissue: Beiging and Metabolic Adaptations. Diabetes. 2015;64:2361–8.

[26] Stallknecht B, Vinten J, Ploug T, et al. Increased activities of mitochondrial enzymes in white adipose tissue in trained rats. Am J Physiol 1991;261:E410–4.

[27] Chechi K, Nedergaard J, Richard D. Brown adipose tissue as an anti-obesity tissue in humans. Obes Rev. 2014;15:92–106.

[28] Welly RJ, Liu TW, Zidon TM, et al. Comparison of Diet vs. Exercise on Metabolic Function & Gut Microbiota in Obese Rats. Med Sci Sports Exerc. 2016.

[29] Koch LG, Britton SL. Artificial selection for intrinsic aerobic endurance running capacity in rats. Physiol Genomics. 2001;5:45–52.

[30] Noland RC, Thyfault JP, Hennes ST, et al. Artificial selection for high-capacity endurance running is protective against high-fat diet-induced insulin resistance. Am J Physiol Endocrinol Metab. 2007;293:E31–41.

[31] Morris EM, Jackman MR, Johnson GC, et al. Intrinsic aerobic capacity impacts susceptibility to acute high-fat diet-induced hepatic steatosis. Am J Physiol Endocrinol Metab. 2014;307:E355–64.

[32] Koch LG, Britton SL, Wisloff U. A rat model system to study complex disease risks, fitness, aging, and longevity. Trends in cardiovascular medicine. 2012;22:29–34.

[33] Wisloff U, Najjar SM, Ellingsen O, et al. Cardiovascular risk factors emerge after artificial selection for low aerobic capacity. Science. 2005;307:418–20.

[34] Crissey JM, Padilla J, Vieira-Potter VJ, et al. Divergent role of nitric oxide in insulin-stimulated aortic vasorelaxation between low- and high-intrinsic aerobic capacity rats. Physiological reports. 2015;3.

[35] Park YM, Kanaley JA, Padilla J, et al. Effects of intrinsic aerobic capacity and ovariectomy on voluntary wheel running and nucleus accumbens dopamine receptor gene expression. Physiol Behav. 2016;164:383–9.

[36] Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care. 1999;22:1462–70.

[37] Padilla J, Jenkins NT, Vieira-Potter VJ, et al. Divergent phenotype of rat thoracic and abdominal perivascular adipose tissues. Am J Physiol Regul Integr Comp Physiol. 2013;304:R543–52.

[38] Xita N, Papasotiropiou I, Georgiou I, et al. The adiponectin-to-leptin ratio in women with polycystic ovary syndrome: relation to insulin resistance and proinflammatory markers. Metabolism. 2007;56:766–71.

[39] Inoue M, Maehata E, Yano M, et al. Correlation between the adiponectin-leptin ratio and parameters of insulin resistance in patients with type 2 diabetes. Metabolism. 2005;54:281–6.

[40] Thyfault JP, Rector RS, Uptergrove GM, et al. Rats selectively bred for low aerobic capacity have reduced hepatic mitochondrial oxidative capacity and susceptibility to hepatic steatosis and injury. J Physiol. 2009;587:1805–16.

[41] Rector RS, Thyfault JP, Morris RT, et al. Daily exercise increases hepatic fatty acid oxidation and prevents steatosis in Otsuka Long-Evans Tokushima Fatty rats. Am J Physiol Gastrointest Liver Physiol. 2008;294:G619–26.

[42] Lovejoy JC, Champagne CM, de Jonge L, et al. Increased visceral fat and decreased energy expenditure during the menopausal transition. Int J Obes (Lond). 2008;32:949–58.
[43] Ruegsegger GN, Booth FW. Health Benefits of Exercise. Cold Spring Harbor perspectives in medicine. 2017.

[44] Oliveira AG, Carvalho BM, Tobar N, et al. Physical exercise reduces circulating lipopolysaccharide and TLR4 activation and improves insulin signaling in tissues of DIO rats. Diabetes. 2011;60:784–96.

[45] Stephenson EJ, Lessard SJ, Rivas DA, et al. Exercise training enhances white adipose tissue metabolism in rats selectively bred for low- or high-endurance running capacity. Am J Physiol Endocrinol Metab. 2013;305: E429–38.

[46] Bluher M. Adipose tissue dysfunction contributes to obesity related metabolic diseases. Best Pract Res Clin Endocrinol Metab. 2013;27:163–77.

[47] Pettersson US, Walden TB, Carlsson PO, et al. Female mice are protected against high-fat diet induced metabolic syndrome and increase the regulatory T cell population in adipose tissue. PLoS One. 2012;7: e46057.

[48] Choi EJ, Jung JY, Kim GH. Genistein inhibits the proliferation and differentiation of MCF-7 and 3T3-L1 cells via the regulation of ERalpha expression and induction of apoptosis. Exp Ther Med. 2014;8:454–8.

[49] Stubbins RE, Holcomb VB, Hong J, et al. Estrogen modulates abdominal adiposity and protects female mice from obesity and impaired glucose tolerance. Eur J Nutr. 2012;51:861–70.

[50] Gollisch KS, Brandauer J, Jessen N, et al. Effects of exercise training on subcutaneous and visceral adipose tissue in normal- and high-fat diet-fed rats. Am J Physiol Endocrinol Metab. 2009;297:E495–504.

[51] Sutherland LN, Bomhof MR, Capozzi LC, et al. Exercise and adrenaline increase PGC-1α mRNA expression in rat adipose tissue. J Physiol. 2009;587:1607–17.

[52] Peppler WT, Anderson ZG, MacRae LM, et al. Habitual physical activity protects against lipopolysaccharide-induced inflammation in mouse adipose tissue. Adipocyte. 2017;6:1–11.

[53] Vieira-Potter VJ, Zidon TM, Padilla J. Exercise and Estrogen Make Fat Cells “Fit”. Exerc Sport Sci Rev. 2015;43:172–8.

[54] Palmer BF, Clegg DJ. The sexual dimorphism of obesity. Mol Cell Endocrinol. 2015;402:113–9.

[55] Park YM, Rector RS, Thyfault JP, et al. Effects of ovariectomy and intrinsic aerobic capacity on tissue-specific insulin sensitivity. Am J Physiol Endocrinol Metab. 2016;310:E190–9.

[56] Waters RP, Renner KJ, Pringle RB, et al. Selection for aerobic capacity affects corticosterone, monoamines and wheel-running activity. Physiology & behavior. 2008;93:1044–54.

[57] Turer AT, Scherer PE. Adiponectin: mechanistic insights and clinical implications. Diabetologia. 2012;55:2319–26.

[58] Maffei M, Halaas J, Ravussin E, et al. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat Med. 1995;1:1155–61.

[59] Tilg H, Moschen AR. Adipokytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol. 2006;6:772–83.

[60] Fernandez-Riejos P, Najib S, Santos-Alvarez J, et al. Role of leptin in the activation of immune cells. Mediators of inflammation. 2010;2010:568343.

[61] Zoth N, Weigt C, Laudenbach-Leschowski U, et al. Physical activity and estrogen treatment reduce visceral body fat and serum levels of leptin in an additive manner in a diet induced animal model of obesity. J Steroid Biochem Mol Biol. 2010;122:100–5.