Effects of exercise and low-fat diet on adipose tissue inflammation and metabolic complications in obese mice

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Submitted 28 January 2009; accepted in final form 5 March 2009

Vieira VJ, Valentine RJ, Wilund KR, Antao N, Baynard T, Woods JA. Effects of exercise and low-fat diet on adipose tissue inflammation and metabolic complications in obese mice. Am J Physiol Endocrinol Metab 296: E1164–E1171, 2009. First published March 10, 2009; doi:10.1152/ajpendo.00054.2009.—Adipose tissue inflammation causes metabolic disturbances, including insulin resistance and hepatic steatosis. Exercise training (EX) may decrease adipose tissue inflammation, thereby ameliorating such disturbances, even in the absence of fat loss. The purpose of this study was to 1) compare the effects of low-fat diet (LFD), EX, and their combination on inflammation, insulin resistance, and hepatic steatosis in high-fat-diet-induced obese mice and 2) determine the effect of intervention duration (i.e., 6 vs. 12 wk). C57BL/6 mice (n = 109) fed a 45% fat diet (HFD) for 6 wk were randomly assigned to an EX (treadmill: 5 days/wk, 6 or 12 wk, 40 min/day, 65–70% VO2max) or sedentary (SED) group. Mice remained on HFD or were placed on a 10% fat diet (LFD) for 6 or 12 wk. Following interventions, fat pads were weighed and expressed relative to body weight; hepatic steatosis was assessed by total liver triglyceride and insulin resistance by HOMA-IR and glucose AUC. RT-PCR was used to determine adipose gene expression of MCP-1, F4/80, TNF-α, and leptin. By 12 wk, MCP-1, F4/80, and TNF-α mRNA were reduced by EX and LFD. Exercise (P = 0.02), adiposity (P = 0.03), and adipose F4/80 (P = 0.02) predicted reductions in HOMA-IR (r2 = 0.75, P < 0.001); only adiposity (P = 0.04) predicted improvements in hepatic steatosis (r2 = 0.51, P < 0.001). Compared with LFD, EX attenuated increases in adiposity, hepatic steatosis, and adipose MCP-1 expression from 6 to 12 wk. There are unique metabolic consequences of a sedentary lifestyle and HFD that are most evident long term, highlighting the importance of both EX and LFD in preventing obesity-related metabolic disturbances.

Visceral white adipose tissue (WAT) is currently believed to be the key depot linked with obesity-related systemic metabolic disturbances (6). WAT becomes inflamed during adipose tissue hypertrophy due to an influx of macrophages (MΦs) that secrete proinflammatory cytokines, including tumor necrosis factor (TNF)-α. The cause of MΦ influx into WAT is not completely understood, but an increase in the gene expression of monocyte chemoattractant protein-1 (MCP-1) in WAT has been shown to precede MΦ entry, suggesting that this chemokine plays an important role in WAT MΦ accumulation (15).

Attenuating inflammation in WAT beneficially modifies (15) these metabolic disturbances and reduces disease risk, even in the absence of obesity reduction (29). Exercise training (EX) has been shown to decrease chronic low-level systemic inflammation in humans (23, 35, 38) as well as improve insulin resistance and hepatic steatosis (8). Unfortunately, the direct effects of EX, with or without low-fat diet (LFD), on WAT inflammation in HFD-induced obesity have not been adequately investigated. Recently, EX was reported to reduce human subcutaneous WAT inflammation (5); however, a limitation of that study was that the visceral depot was not assessed (10). Animal models have shown that EX (treadmill running) lowers WAT inflammation in the visceral of nonobese animals (9, 26), suggesting that exercise may be a useful therapy. Furthermore, Bradley et al. (3) demonstrated that voluntary wheel running improved insulin resistance and decreased visceral WAT inflammation in mice simultaneously fed a HFD. Although that study suggests that exercise is effective in improving insulin resistance and decreasing WAT, those animals ran more than 10 km/day, which is difficult to translate to humans. Therefore, it is unknown whether moderate levels of EX improve visceral WAT inflammation in a model of HFD-induced obesity. To date, no studies have investigated the duration effects (i.e., short-term vs. long-term effects) of caloric reduction and exercise in terms of fat loss, metabolic complications, and WAT inflammation.

The purpose of the present study was to investigate the effects of 6 and 12 wk of EX and LFD on WAT inflammation and metabolic disturbances using HFD-induced obese C57BL/6 mice. We hypothesized that LFD would more robustly reduce body weight and fat content compared with a moderate-intensity exercise program. Yet the effects of the interventions on WAT inflammation and metabolic complications would be similar. We hypothesized that the effects of a LFD would occur earlier than the effects of EX (i.e., at 6 wk) but that the effects of EX will be evident by 12 wk. Moreover, we hypothesized that the combination of treatments would be better than either individual treatment at both intervention durations.
**METHODS**

*Animals and diets.* Male C57BL/6 mice (n = 109) were purchased from Jackson Laboratories (Bar Harbor, ME) at 4 wk of age. After a 1-wk acclimation period, all mice were individually caged and switched to a 45% fat diet (HFD) for 6 wk to induce obesity and then were randomly assigned to HFSED (HFD, sedentary), HFEX (HFD, exercised), LFSED [10% fat diet (LFD), no sedentary], or LFEX (LFD, exercise) groups for a 6- or 12-wk period. The HF and LFD were purchased from Research Diets (New Brunswick, NJ); more specific information regarding nutrient composition can be found in Table 1. All groups were allowed to eat ad libitum, and food intake and body weight were recorded twice/wk for the duration of the study. The animals were housed on a reverse light-dark cycle (lights on at 2100 and off at 0900) at 25°C. National Institutes of Health guidelines for the care and use of laboratory animals were strictly followed, and all experiments were approved by the Animal Care and Use Committee at the University of Illinois at Urbana-Champaign.

**EX protocol.** Animals were exercise trained (EX) during their dark cycle (i.e., during their active period) between 0900 and 1200 on a motorized treadmill (Jog-a-Dog, Ottawa Lake, MI) for 40 min/day at cycle (i.e., during their active period) between 0900 and 1200 on a treadmill at the University of Illinois at Urbana-Champaign. All groups were allowed to eat ad libitum, and food intake and body weight were recorded twice/wk for the duration of the study. The animals were housed on a reverse light-dark cycle (lights on at 2100 and off at 0900) at 25°C. National Institutes of Health guidelines for the care and use of laboratory animals were strictly followed, and all experiments were approved by the Animal Care and Use Committee at the University of Illinois at Urbana-Champaign.

The intensity corresponded to 12 m/min, 12% grade, 5 times/wk, for 6 or 12 wk; this intensity was maintained using a motorized treadmill (Jog-a-Dog, Ottawa Lake, MI) for 40 min/day at cycle (i.e., during their active period) between 0900 and 1200 on a treadmill at the University of Illinois at Urbana-Champaign. All groups were allowed to eat ad libitum, and food intake and body weight were recorded twice/wk for the duration of the study. The animals were housed on a reverse light-dark cycle (lights on at 2100 and off at 0900) at 25°C. National Institutes of Health guidelines for the care and use of laboratory animals were strictly followed, and all experiments were approved by the Animal Care and Use Committee at the University of Illinois at Urbana-Champaign.

**Table 1. Diet composition**

<table>
<thead>
<tr>
<th></th>
<th>HFD%</th>
<th>LFD%</th>
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<tr>
<td>Protein</td>
<td>24</td>
<td>19.2</td>
<td>20</td>
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<tr>
<td>CHO</td>
<td>41</td>
<td>35</td>
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<tr>
<td>Fat</td>
<td>24</td>
<td>4.3</td>
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<tr>
<td>kcal/g</td>
<td>4.73</td>
<td>3.85</td>
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**HFD, high-fat diet; LFD, low-fat diet; CHO, carbohydrate. Rodent diets D12450B and D12451; Research Diets (New Brunswick, NJ).**

**Statistical analysis.** Body weight (BW) changes throughout the 6- and 12-wk interventions were analyzed using a one-way analysis of variance (ANOVA) with repeated measurements; post hoc Tukey HSD comparisons were used to determine individual group differences when a significant F ratio was obtained. Differences in initial BW, final BW, final organ weights, fasting glucose and insulin, glucose AUC, hepatic TG content, HOMA-IR, plasma SAA, plasma adiponectin, food intake, and WAT mRNA levels were determined using one-way ANOVA with post hoc Tukey comparisons where appropriate. Prevalence of HS was assessed using chi-square analysis. Duration effects were assessed using a univariate three-way ANOVA with EX (EX or SED), LFH (LF or LFD), and duration (6 vs. 12 wk) as between-subjects factors; interaction effects were also determined. To determine whether treatment effects were independent of relative epididymal adiposity, the epididymal fat pad/BW ratio was used as a covariate. Correlation values were Pearson’s bivariate. Where applicable, linear regression analyses were used. All analyses were done using SPSS V15 (SPSS, Chicago, IL), and data are presented as means ± SE unless noted otherwise. α-Level for main effects was set at P ≤ 0.05.
RESULTS

**EX and LFD attenuate BW and WAT gain in obese mice.** After 6 wk of HFD feeding, there were no preintervention group differences in BW between mice randomized to the 6-wk or 12-wk intervention (Table 2). Compared with all other groups, HFSED gained more weight over the course of either the 6- (P < 0.001) or 12-wk (P < 0.001) interventions. Among the three interventions, LFEX gained less weight than HFXE (P < 0.05) but not LFSED after either length intervention. Epididymal fat pad weight was also reduced by the interventions (P < 0.05). Only after the 12-wk intervention was there an additive benefit of the combined treatment, as LFEX exhibited significantly lower epididymal fat pad weight compared with HFXE or LFSED (Table 2). Similar results were found when epididymal fat pad weight was expressed as relative to BW (Table 2) and for retroperitoneal fat pad weight (data not shown). Heart weight/BW ratio [a general indicator for an aerobic training effect (14)] tended (P = 0.08) to be elevated in the intervention groups after 12 wk of intervention. At 6 wk, there was a group main effect for caloric intake; HFSED consumed more calories than both LFD groups. In response to the 12-wk intervention, HFSED consumed more calories than both LFD groups, and the HFXE group consumed more calories than LFSED but not LFEX (Table 2). In no instance did any mouse exhibit overt splenomegaly or symptoms (e.g., reduced weight, food intake, grooming behavior, or nest building) indicative of acute infection, nor were there any visible tumors upon necropsy.

**Different treatment effects on insulin sensitivity.** After the 6-wk intervention, fasting blood glucose was reduced in HFXE compared with HFSED (Table 3). By 12 wk, both EX groups exhibited lower fasting blood glucose compared with HFSED, and the combined intervention resulted in lower glucose compared with LFD alone. In contrast, 6-wk fasting insulin values were lower in response to all interventions compared with HFSED (P < 0.05). By 12 wk, both LFD groups, but not HFEX, had lower fasting insulin vs. HFSED (P < 0.05). As a result, HOMA-IR was lower in all intervention groups after the 6-wk intervention but only in the LFD groups after 12 wk of intervention. There was a duration effect (P = 0.009), but not a group × duration effect (P = 0.57), such that HOMA-IR increased over time in all groups. GTT revealed that after 6 wk of intervention both LFD groups, but not HFEX, were better able to reduce glucose AUC (P < 0.001). LFEX was also more efficacious compared with EX alone (i.e., HFEX). After 12 wk of intervention, both LFD interventions reduced glucose AUC (P < 0.001) compared with groups ingesting HFD (Table 3). There were no duration or treatment × duration effects for glucose AUC. Regression analyses revealed that F4/80 WAT gene expression was most strongly related to HOMA-IR at 12 wk (β = 0.83, P < 0.001; regression r² = 0.75; F₄,₄₂ = 29.1, P < 0.001). Adiposity (β = 0.56, P < 0.001), HFD (β = 0.27, P = 0.05), and lack of EX (i.e., SED) (trend β = 0.22, P = 0.06) were also associated with HOMA-IR.

Exercise, but not LFD, attenuates obesity-related hepatic steatosis. There were no between-group differences in liver weight in response to 6-wk intervention (0.82 ± 0.06, 0.83 ± 0.06, 0.83 ± 0.06, and 0.72 ± 0.06 for HFSED, HFXE, LFSED, and LFEX, respectively). In response to the 12-wk intervention, although all interventions reduced liver weight (1.2 ± 0.10, 0.91 ± 0.05, 0.96 ± 0.04, and 0.92 ± 0.06 for HFSED, HFXE, LFSED, and LFEX, respectively; P = 0.02), only HFEX and LFEX did so significantly relative to HFSED. At 6 wk, there were differences in liver triglycerides (Fig. 1) such that LFEX was lower than HFSED and HFXE (P = 0.001). In contrast, at 12 wk, both exercise groups (HFXE and LFEX) exhibited lower liver TG values compared with the SED groups (P < 0.001). Regression analysis revealed adiposity (β = 0.73, P = 0.001; regression r² = 0.63; F₅,₄₂ = 12.6, P < 0.001) to be the strongest correlate of hepatic steatosis, independent of F4/80 (P = 0.70), SED (P = 0.69), and HOMA-IR (P = 0.12). Consumption of LFD (β = 0.53, P = 0.004) was also positively related to steatosis.

When comparing outcomes between the 6- and 12-wk durations, we found a treatment by duration effect (P = 0.03), indicating that HFD feeding from 6 to 12 wk resulted in an additional increase in hepatic steatosis that was completely eliminated when mice were exercised, regardless of diet (Fig. 1). Clinically significant hepatic steatosis is defined as liver TG exceeding 5% of liver weight (2). Based upon this definition, when we compared the percentages of steatotic mice between treatment groups, we found that EX (χ² = 9.5, P = 0.002), but not LFD (χ² = 2.2, P = 0.12), significantly reduced the incidence of hepatic steatosis in response to the 12- but not 6-wk intervention.

Exercise and diet intervention reduces systemic inflammation, and exercise reduces adiponectin in HFD-fed mice. In a random subset of mice (n = 9–10/group), we found that the systemic inflammatory marker SAA was lowered by all interventions (P = 0.003), with no differences between interven-

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**Table 2. Body weight, fat pad weight, and caloric intake in response to 6- or 12-wk interventions**

<table>
<thead>
<tr>
<th></th>
<th>HFSED</th>
<th>HFXE</th>
<th>LFSED</th>
<th>LFEX</th>
<th>HFSED</th>
<th>HFXE</th>
<th>LFSED</th>
<th>LFEX</th>
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<tr>
<td><strong>6-Wk Intervention</strong></td>
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<tr>
<td>n</td>
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<td>15</td>
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<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Initial BW, g</td>
<td>25.8±0.4</td>
<td>24.8±0.4</td>
<td>25.5±0.4</td>
<td>25.0±0.5</td>
<td>24.7±0.7</td>
<td>24.8±0.6</td>
<td>25.0±0.8</td>
<td>25.0±0.7</td>
</tr>
<tr>
<td>Final BW, g</td>
<td>32.1±0.9</td>
<td>28.2±0.7b</td>
<td>26.9±0.3c</td>
<td>24.9±0.4</td>
<td>38.5±1.2**</td>
<td>34.0±1.2b*</td>
<td>30.7±0.8b,c*</td>
<td>29.4±0.6*</td>
</tr>
<tr>
<td>Epididymal fat weight, g</td>
<td>1.8±0.2</td>
<td>1.0±0.1b</td>
<td>0.7±0.03c</td>
<td>0.5±0.03c</td>
<td>2.1±0.08**</td>
<td>1.5±0.2b*</td>
<td>1.1±0.1b,a</td>
<td>0.6±0.05*</td>
</tr>
<tr>
<td>REA, %</td>
<td>5.4±0.3b</td>
<td>3.6±0.4b</td>
<td>2.6±0.1b,c</td>
<td>2.1±0.1c</td>
<td>5.6±0.2a*</td>
<td>4.3±0.4</td>
<td>3.6±0.2ab*</td>
<td>2.0±0.1c</td>
</tr>
<tr>
<td>Heart weight/BW</td>
<td>0.65±0.08</td>
<td>0.84±0.2</td>
<td>0.65±0.05</td>
<td>0.75±0.07</td>
<td>0.40±0.04*</td>
<td>0.51±0.05</td>
<td>0.54±0.04</td>
<td>0.60±0.06</td>
</tr>
<tr>
<td>Cumulative caloric intake, kcal</td>
<td>512±16b</td>
<td>474±12b</td>
<td>455±14b</td>
<td>426±8b</td>
<td>1039±23a</td>
<td>994±20ab*</td>
<td>916±18a</td>
<td>936±13b,c*</td>
</tr>
</tbody>
</table>

Values are means ± SE. HFSED, HFD, sedentary; HFXE, HFD, exercised; LFSED, 10% fat diet (LFD), no sedentary; LFEX, LFD, exercise; n, no. of animals; BW, body weight; REA, epididymal fat pad weight/BW. Different letters represent significant between-group differences within time point based on ANOVA with post hoc Tukey’s test. *Significant duration effect within group.
Adiponectin levels were not different between groups at the 6-wk time point. At 12 wk, we found a group effect such that both EX groups were lower than both SED groups ($P < 0.001$). There was a duration effect on adiponectin levels such that adiponectin levels decreased over time in the intervention groups ($P < 0.001$; Fig. 2B).

Effects of exercise and LFD intervention on WAT adipokine gene expression. The housekeeping gene GAPDH was used to correct mRNA expression values for all genes examined, and there were no intervention-induced differences in GAPDH (data not shown). At 6 wk, there was a difference ($P = 0.003$) in WAT leptin gene expression such that LFEX was lower than HFSED ($P = 0.05$; Fig. 3A). In contrast, by 12 wk both LFSED and LFEX leptin mRNA levels were lower than HFSED ($P < 0.05$). Exercising while on a HFD tended to result in lower WAT leptin mRNA expression ($P = 0.09$). There were no intervention duration effects for WAT leptin mRNA expression.

WAT MCP-1 mRNA expression was not significantly ($P = 0.06$) affected by the 6-wk intervention; however, EX and LFD interventions resulted in lower MCP-1 expression in WAT by 12 wk ($P < 0.001$) (Fig. 3B). Moreover, there was an intervention duration interaction effect ($P < 0.001$) such that WAT MCP-1 mRNA expression was higher at 12 wk compared with 6 wk in the HFSED but not the other groups (Fig. 3B).

WAT F4/80 mRNA expression (indicative of $M\phi$ presence) was affected by the 6-wk intervention such that both LFD interventions resulted in lower WAT F4/80 mRNA expression compared with both SED interventions ($P < 0.05$). There were no intervention duration effects for WAT F4/80 mRNA expression.

Values are means ± SE. HOMA-IR, homeostatic model of insulin resistance; AUC, area under the curve. All variables except glucose AUC were measured in the fasted state (12 h of no food). Different letters represent significant between-group differences within time point based on ANOVA with post hoc Tukey’s test. *Significant duration effect within group.

Table 3. Fasting blood glucose, insulin, HOMA-IR, and glucose AUC

<table>
<thead>
<tr>
<th></th>
<th>6-Wk Intervention</th>
<th>12-Wk Intervention</th>
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<tr>
<td></td>
<td>HFSED</td>
<td>HFEX</td>
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<tr>
<td>n</td>
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<td></td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>13.27±0.6a</td>
<td>10.51±0.30b</td>
</tr>
<tr>
<td>Insulin, μIU/ml</td>
<td>15.95±1.8a</td>
<td>10.23±0.4b</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>9.41±1.2a</td>
<td>4.77±0.2b</td>
</tr>
<tr>
<td>Glucose AUC</td>
<td>49,563±800a</td>
<td>42,210±2.243b</td>
</tr>
</tbody>
</table>

Fig. 1. Effect of a 6- or 12-wk exercise or low-fat diet intervention on liver triglyceride values in obese mice. Different letter designations represent significant ($P < 0.05$) group differences within 6- or 12-wk intervention. *Significant within-treatment group difference at 12- vs. 6-wk intervention. Data are means ± SE; $n = 12$–15 mice/group. HFSED, high-fat diet, sedentary; HFEX, high-fat diet, exercised; LFSED, 10% fat diet (low-fat diet), no sedentary; LFEX, low-fat diet, exercise.

Fig. 2. Effects of 6- or 12-wk intervention on serum amyloid A (A) and serum adiponectin (B). Different letters represent significant between-group differences within 6- or 12-wk intervention. *Significant within-treatment group difference at 12- vs. 6-wk intervention. Data are means ± SE; $n = 9$–10/group.

Fig. 3. Effects of 6- or 12-wk intervention on WAT adipokine gene expression. A) Leptin, B) MCP-1, C) F4/80.
groups expressed less F4/80 mRNA compared with HFSED ($P = 0.004$) but not HFEX (Fig. 3C). By 12 wk of intervention, all EX and LFD interventions exhibited lower ($P = 0.001$) F4/80 mRNA compared with HFSED. As was the case with WAT MCP-1 expression, F4/80 expression also increased ($P = 0.001$) at the 12-wk time point (vs. 6 wk) in the HFSED but not the other groups (Fig. 3C).

In response to 6 wk of intervention, LFD, but not EX, reduced WAT TNF-α gene expression; LFEX had lower ($P = 0.04$) TNF-α than HFEX (Fig. 3D). In stark contrast, after 12 wk of intervention both LFD and EX lowered TNF-α mRNA expression in visceral WAT ($P < 0.001$). Similar to MCP-1 and F4/80 gene expression, continued HFD feeding from 6 to 12 wk led to further increases in TNF-α mRNA (duration effect, $P = 0.002$). This increase was abolished in response to either EX, LFD, or their combination (Fig. 3D).

**DISCUSSION**

The most important finding of this study was that moderate exercise (EX), LFD, or their combination resulted in a significant attenuation of HFD-induced increases in systemic and WAT inflammation. These effects were stronger after 12 wk compared with 6 wk. At 6 wk, only the combined treatment reduced adiposity, leptin gene expression, and hepatic steatosis. Both LFD interventions reduced adipose F4/80 gene expression, and all three interventions improved insulin resistance. By 12 wk, all interventions reduced adiposity, circulating SAA, and adipose F4/80, MCP-1, and TNF-α gene expression. Interestingly, LFD had a more robust ability to reduce adiposity and insulin resistance, yet the effects of the individual treatments on inflammatory outcomes were similar. These findings demonstrate that both EX and LFD play important roles in reducing WAT inflammation and the associated metabolic disturbances and that the mechanisms responsible for their effects may be different.

It has recently been reported that accumulation of visceral WAT is causally related to metabolic outcomes (6), a relationship presumably due to the inflammatory processes that take place in obese WAT. In this study, both LFD and EX attenuated body weight (BW) gain and adiposity after the initial 6-wk HFD feeding period, although the LFD had a more vigorous effect. Interestingly, by 12 wk, LFD and EX had equal and additive effects on adiposity. Furthermore, the only significant increase in relative adiposity from 6 to 12 wk was in the LFSED group, suggesting that EX effectively attenuated adipose tissue accretion in the viscera, whereas LFD in isolation did not. Studies have concluded that EX has important benefits over diet alone, specifically in terms of its effects on preferentially reducing visceral adipose (30), which our findings support. Combining the moderate exercise program with a reduction in caloric intake was more robust than either single intervention, supporting the current public health guidelines suggesting that Americans perform at least moderate amounts of physical activity (1) and reduce their saturated fat intake to less than 10% of total calories (31).

Similar to the stronger fat-reducing effects of LFD compared with EX at 6 wk, only LFD resulted in lower epididymal WAT F4/80 and TNF-α gene expression. However, the more robust fat-reducing effects of LFD early on may have resulted in this intervention having a more profound effect on WAT inflammation. By 12 wk both LFD and EX reduced MCP-1, F4/80, and TNF-α, suggesting that by 12 wk EX was just as effective as LFD in decreasing relative adiposity and WAT inflammation. Furthermore, it seems that, by the end of the extended intervention, both LFD and EX lowered WAT inflammation by mechanisms that were not fully explained by reduction in visceral fat. That is, when statistical adjustments were made for the effect of adiposity reduction, both interventions still lowered inflammation (data not shown). To determine the kinetics of WAT inflammation in obesity and how EX and LFD may mitigate this,
we examined the effect of intervention duration on inflammatory markers in the WAT. Compared with HFSED, all three interventions completely prevented the inflammatory exacerbation from 6 to 12 wk (MCP-1, F4/80, TNF-α), suggesting that all interventions were equally effective at attenuating adipose tissue inflammation.

The increase in WAT inflammation in obesity contributes to systemic inflammation (18), as measured by C-reactive protein (4) and SAA (39). Indeed, we found significant correlations between SAA and F4/80 (r = 0.8, P < 0.001), MCP-1 (r = 0.4, P < 0.01), and TNF-α (r = 0.7, P < 0.001). These findings support other work showing WAT MΦ infiltration to be a causative factor in systemic inflammation and its metabolic consequences (22). Although there were no group differences in circulating levels of SAA at 6 wk, all treatments effectively reduced SAA by 12 wk, an effect that was reduced when adiposity was adjusted (data not shown), suggesting that fat loss contributed to this reduction in circulating inflammation.

Adiponectin levels are usually inversely related to insulin resistance and inflammation and increase with weight loss and/or exercise (21). Quite surprisingly, in this study, EX decreased circulating levels of adiponectin at 12 wk, which remained significant after the effect of adiposity was adjusted, suggesting that EX lowered adiponectin levels at 12 wk by a mechanism unrelated to changes in visceral adipose tissue. Moreover, adiponectin levels decreased from 6 to 12 wk in all interventions. Studies on the effects of EX on circulating adiponectin have been equivocal (24), with most studies showing a beneficial (i.e., increasing) effect of fat loss, but not exercise per se (13), and some showing additional benefits of vigorous exercise (7, 25). No previous studies have reported an adiponectin-reducing effect of exercise, but no studies have investigated how adiponectin levels differ at different-length exercise interventions in mice. More research is necessary to definitively determine the unique effects of EX and LFD on adiponectin as well as the implications and mechanisms behind these effects.

At 6 wk, all interventions were equally effective at improving HOMA-IR, but only LFD significantly improved glucose tolerance. By 12 wk, LFD (but not EX) improved HOMA-IR and glucose tolerance. This may have been attributed to the better fat-reducing effect of LFD compared with EX. Cytokines produced by WAT (11) as well as the MΦs that reside there (22) have been shown to interfere with insulin signaling, causing insulin resistance. In support of this idea, regression analyses revealed F4/80 WAT gene expression to be most strongly related to HOMA-IR at 12 wk.

In contrast to the results on insulin resistance, although both treatments reduced hepatic steatosis at 6 wk, only EX effectively did so by 12 wk. Previous studies have shown that weight loss (20) and EX (8) reduce hepatic steatosis, whereas high-carbohydrate diets (such as the LFD used here, which is 70% sucrose) may increase hepatic steatosis by promoting de novo lipogenesis (12). Here we also report that continued consumption of the LFD was related to an increase in hepatic steatosis, because there was a significant effect of intervention duration such that only the LFSED group experienced an increase from 6 to 12 wk. This is unlike studies that have shown that switching from a HFD to a normal rodent chow diet reverses hepatic steatosis; the lack of such an effect in this study is probably due to the high sucrose content of the LFD compared with standard rodent chow. The LFD used in this study has been used as a high-carbohydrate diet in other studies (37). Importantly, the current study illustrates that EX, regardless of diet, is an effective means to prevent hepatic steatosis. The fact that EX did not affect insulin resistance, but did improve hepatic steatosis at 12 wk, may suggest that hepatic steatosis is affected before insulin resistance. This is an important finding because no studies have yet identified the time course with which these metabolic disturbances occur and/or are reduced with behavioral interventions. Our findings are in support of recent human studies showing visceral adipose accumulation to be predictive of hepatic steatosis (33). Regression analysis at 12 wk revealed that relative visceral adiposity was the most important correlate of hepatic steatosis, independent of HOMA-IR and WAT expression of F4/80, MCP-1, and TNF-α. This suggests that other factors residing in, or related to, WAT that were not measured in this study may also be important mediators of hepatic steatosis.

It has been shown that diets high in saturated fats directly cause WAT inflammation, and the mechanism is thought to involve Toll-like receptor signaling (36) and/or MΦ phenotype switching toward an inflammatory profile (17), which may help explain the independent effects of LFD on the inflammatory and metabolic outcomes reported here. The finding that EX has unique anti-inflammatory effects is novel and likely will have important implications, given the strong independent relationship between WAT inflammation and obesity-related comorbidities (33) and the lack of long-term success of dietary interventions without exercise (34). Mechanisms by which EX reduces WAT inflammation may involve improvements in the “health” of the WAT, including reduced adipocyte size, increased blood flow, increased mitochondrial function and facilitated fatty acid oxidation, decreased cellular stress, and/or improved resistance to cell stress. Recent studies have implicated hypoxia as a cause of adipose tissue inflammation in obesity (40). Although no studies have yet determined whether exercise decreases hypoxia in obese WAT, it is plausible that an exercise-related increase in WAT blood flow may mediate its anti-inflammatory effects on WAT.

The findings presented here should be considered along with this study’s limitations. The mode of EX used in this study was treadmill running, which has the disadvantage of the stress associated with its forced nature (although our control animals were yoked). Treadmill training has significant advantages over voluntary wheel running (which could be considered an observational intervention) because the intensity and duration of the running can be accurately dosed. When given a running wheel, some mice partake in extreme physical activity (>10 km/day), and the variability in the amount run between mice is high and difficult to control. Thus, the public health generalizability of such doses (e.g., 10 km) of exercise could be questioned. We feel that treadmill running is an appropriate model to examine the influence of exercise. More studies where fat loss is strictly matched in LFD and EX groups to definitively test the hypothesis that EX and LFD have adipose tissue loss-independent effects are necessary. Also, because tissues needed to be extracted for the studies to be carried out, the ideal repeated-measures design, where the same animals were used at both time points, was not an option. Thus, the analyses of intervention duration were done using different sets of mice. Finally,
although F4/80 gene transcription is often used as an indicator of MΦ presence (28), direct assessment of WAT MΦ content is needed.

In summary, the current findings confirm previous data regarding the potential anti-inflammatory effect of EX and contribute insight regarding the time course with which these interventions improve obesity and its related metabolic complications. Although at 6 wk of intervention improvements in metabolic disturbances and WAT inflammation were likely attributed to reductions in relative adiposity, by 12 wk independent treatment effects were observed such that both EX and LFD independently reduced WAT inflammation. EX improved hepatic steatosis, whereas LFD improved glucose tolerance and HOMA-IR. LFD caused more robust reductions in relative adiposity, whereas hepatic steatosis increased on a LFD. Taken together, these findings suggest that LFD, EX, and visceral WAT reduction all impart anti-inflammatory properties to the WAT and improve metabolic outcomes.

ACKNOWLEDGMENTS
We thank Drs. Rodney Johnson and Kimberly Huey for assistance with the conduct of the study.

GRANTS
This research was supported by National Institute on Aging Grant AG-023580 and an American College of Sports Medicine doctoral student research grant to V. J. Vieira.

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AJP-Endocrinol Metab • VOL 296 • MAY 2009 • www.ajpendo.org

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