Short-term weight loss attenuates local tissue inflammation and improves insulin sensitivity without affecting adipose inflammation in obese mice

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Obesity is a major cause of a multitude of human diseases, including type 2 diabetes, cardiovascular disease, and cancer (7). Although epidemic prevalence of obesity continues to be a major threat to human health, the benefits of lifestyle intervention to deflect this trend are highly encouraging (29). In that regard, weight loss was shown to improve insulin resistance in obese subjects, and this was strongly associated with reduced visceral obesity (24). Weight management program using dietary intervention also improved insulin sensitivity in overweight children (28). Insulin resistance is a multifactorial disease, and its etiology involves dysregulation of lipid metabolism and adipose-derived hormones (2, 26). In that regard, weight loss was shown to increase serum adiponectin levels and enhance lipid oxidation in obese subjects (20, 30). Intrahepatic fat was also shown to be reduced following weight loss mediated by diet intervention, and this was associated with improved insulin sensitivity in liver and skeletal muscle of obese children (31). Furthermore, mitochondrial function in skeletal muscle improved after exercise-mediated weight loss in obese subjects (22). In contrast, circulating levels of resistin, which negatively regulates glucose metabolism, were shown to increase in obese subjects after a 4-mo weight loss intervention (19).

Recent evidence indicates that obesity is a low-grade, chronic inflammatory state, and adipose tissue inflammation plays a major role in obesity-induced insulin resistance (32, 33). Circulating levels of inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor (TNF)α, are elevated in obese diabetic subjects (16). Inflammation develops in multiple organs, and both IL-6 and TNFα have been shown to cause insulin resistance by downregulating the insulin signaling pathway (10, 25). To this end, recent studies have examined the effects of weight loss on inflammation, and their findings are inconsistent. Nicklas et al. (23) found that weight loss with dietary intervention reduced serum levels of IL-6, TNFα, and C-reactive protein in obese subjects. Esposito et al. (6) also found that weight loss induced by dietary and activity intervention decreased serum IL-6, IL-18, and C-reactive protein levels in obese women. In contrast, Christiansen et al. (4) reported no significant effects of exercise-mediated weight loss on inflammatory cytokines in obese subjects.

Taken together, these findings indicate that more studies are clearly needed to determine the underlying mechanism by which weight loss improves insulin sensitivity in obese subjects. The challenges faced in human studies, and part of the reason for inconsistent data, come from difficulty in restricting a subject’s lifestyle in accordance with intervention strategy. Thus, in this study, we used diet intervention and exercise in C57BL/6 mice to examine the molecular effects of weight loss on multiple metabolic parameters, including energy balance and insulin sensitivity and inflammation. Our findings indicate that weight loss generally improves inflammatory state but...
differentially affects insulin sensitivity depending on the modality of weight loss intervention.

**MATERIALS AND METHODS**

**Animals, diet, and exercise.** Male C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed under controlled temperature (23°C) and lighting conditions (12 h of light, 0700–1900; 12 h of dark, 1900–0700). Starting at 8 wk of age, mice were fed a high-fat diet (HFD) (Harlan-Teklad TD 93075; 55% kcal from fat, 24% kcal from carbohydrate, and 21% kcal from protein) ad libitum for 12 wk to induce obesity. A separate cohort of mice were given a low-fat diet (LFD) (LabDiet 5P76, 14% kcal from fat, 60% kcal from carbohydrate, and 26% kcal from protein) for 12 wk to serve as controls (n = 20). At the end of 12 wk of HFD, mice were randomized into three groups (n = 20 mice/group); one group of mice continuously received a HFD for additional 3 wk, another group received a LFD for 3 wk to induce weight loss by diet intervention, and the last group continuously received a HFD plus cage wheel for 3 wk to induce weight loss by exercise. All mice were housed individually throughout the study. This was necessary particularly for the exercise group that required a cage wheel and to prevent competitive feeding/exercise behavior from group housing. Based on a study by Arndt et al. (1), we expected minimal effects of individual housing on mouse behavior or stress. The animal studies were approved by the Institutional Animal Care and Use Committee of the University of Massachusetts Medical School.

**Body composition and energy balance measurement.** The effects of HFD, LFD, and exercise on body composition were monitored by noninvasively measuring whole body fat and lean mass using 1H-MRS (Echo Medical Systems, Houston, TX). We performed a baseline energy balance measurement at the end of 12 wk of HFD and before the start of exercise or LFD switch. We repeated the final energy balance measurement after 3 wk of exercise or LFD switch. The food intake, energy expenditure, respiratory exchange ratio, and physical activity were assessed for 3 days at baseline and at the end of study using metabolic cages (TSE Systems, Chesterfield, MO). We used the TSE Systems LabMaster platform with easy-to-use calorimetry, featuring fully automated monitoring for food and water and XYZ activity. Labmaster cages that are most similar to facility home cages were used, thereby allowing the use of bedding in the cage and minimizing any animal anxiety during the experimental period. The system provides intuitive software with flexibility for experimental setup and data utilization.

**Hyperinsulinemic euglycemic clamp to assess insulin sensitivity in conscious mice.** At 4–5 days before clamp experiments, a survival surgery was performed to establish indwelling catheter in the jugular vein. On the day of the experiment, mice were fasted overnight (~15 h), and a 2-h hyperinsulinemic euglycemic clamp was conducted in conscious mice with a primed and continuous infusion of human insulin (150 mU/kg body wt priming, followed by 2.5 mU · kg⁻¹ · min⁻¹; Novolin, Novo Nordisk) (13). To maintain euglycemia, 20% glucose was infused at variable rates during clamps. Whole body glucose turnover

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**Fig. 1.** Short-term low-fat diet and exercise intervention causes weight loss in mice. Male C57BL/6 mice were fed a low-fat diet (LFD; controls) or high-fat diet (HFD) for 12 wk. On day 0 of weight loss intervention, 1 group of HFD-fed mice was given a cage-running wheel to induce exercise while continuing on a HFD (HFD + EX; n = 20). Another group of HFD-fed mice was given a LFD switch to induce weight loss (HFD → LFD; n = 20). One group of HFD-fed mice remained on a HFD (HFD; n = 20), and controls remained on a LFD (LFD; n = 20). A: longitudinal measurement of body weight during 21 days of weight loss intervention. B and C: whole body fat mass and lean mass, measured using 1H-MRS, during 18 days of diet and exercise intervention.
was assessed with a continuous infusion of \([3-3H]\)glucose (Perkin-Elmer), and 2-deoxy-D-[1-\(^{14}\)C]glucose (2-[\(^{14}\)C]DG) was administered as a bolus (10 \(\mu\)Ci) at 75 min after the start of clamps to measure insulin-stimulated glucose uptake in individual organs. At the end of the clamps, mice were anesthetized, and tissues were taken for biochemical analysis (13).

**Biochemical assays.** Glucose concentrations during clamps were analyzed using 10 \(\mu\)l of plasma by a glucose oxidase method on an Analox GM9 Analyzer (Analox Instruments, Hammersmith, London, UK). Plasma concentrations of \([3-3H]\)glucose, 2-[14C]DG, and \(^3\)H\(_2\)O were determined following deproteinization of plasma samples as previously described (13). For the determination of tissue 2-[\(^{14}\)C]DG-6-phosphate (2-[\(^{14}\)C]DG-6-P) content, tissue samples were homogenized, and the supernatants were subjected to an ion exchange column to separate 2-[\(^{14}\)C]DG-6-P from 2-[\(^{14}\)C]DG. Insulin-stimulated glucose uptake in individual tissues was assessed by determining the tissue content of 2-[\(^{14}\)C]DG-6-P and plasma 2-[\(^{14}\)C]DG profile.

**Molecular analysis of insulin signaling and inflammation.** Muscle samples were obtained at the end of clamps to measure in vivo insulin signaling activity. Immunoblotting was performed using powdered samples that were dissolved in lysis buffer (50 mM HEPES, pH 7.3, 137 mM NaCl, 1 mM MgCl\(_2\), 1 mM CaCl\(_2\), 2 mM NaVO\(_4\), 10 mM sodium pyrophosphate, 10 mM NaF, 2 mM EDTA, 2 mM PMSF, 10 mM benzamidine, 10% glycerol, 1% Triton X-100, 1 mM microcystin LR, 100 nM okadaic acid, and cocktail protease inhibitor) and sonicated for 10 s. The samples were incubated on ice for 30 min and centrifuged at 15,000 \(g\) for 15 min at 4°C, and the supernatants were harvested. Protein concentrations were determined using the BCA Protein Assay Kit (Thermo Scientific). The proteins were dissolved in SDS gel sample buffer and resolved by electrophoresis on 10% polyacrylamide gels. After electrophoresis, proteins were transferred to nitrocellulose membranes (Bio-Rad), blocked in 5% nonfat milk in 50 mM Tris · HCl, pH 7.5, 100 mM NaCl, and 0.1% Tween-20, and incubated with anti-Akt and anti-phospho-Akt (Ser\(^{473}\)) antibodies (1:1,000 dilution; Cell Signaling Technology). The membranes were then incubated in appropriate goat anti-rabbit IgG or goat anti-mouse secondary antibodies conjugated to horseradish peroxidase (1:5,000 dilution; Pierce). Blots were developed in Detection Reagents (Thermo Scientific) and scanned by BioSpectrum (UVP), and the density was quantified by Image Acquisition & Analysis Software (UVP).

For CD68 levels using Western blot, muscle and liver samples were fixed for 24 h at room temperature in Bouin’s solution (Sigma-Aldrich), and the samples were placed into cassettes, which were immersed in 70% EtOH. 70% EtOH was changed several times to wash away the fixative until it became clear. Skeletal muscle sample was embedded in paraffin. Tissue section (5 \(\mu\)m) was mounted on charged glass slides. For deparaffinizing tissue, slide was incubated for 5 min in clean xylene, 100, 95, 85, and 70% EtOH, and ultrapure water. Tissue section was immersed in Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, and 0.05% Tween-20, pH 9.0; Sigma-Aldrich) at 95°C for 20 min to retrieve antigen and cooled at room temperature.

**Fig. 2.** Weight loss affects energy balance in obese mice. A 3-day indirect calorimetry study was performed using TSE metabolic cages after weight loss intervention mediated by LFD or exercise. A: daily food intake. B and C: energy expenditure rates normalized to whole body lean mass during day (7 AM to 7 PM) and night (7 PM to 7 AM) cycle. D: \(\text{VO}_2\) consumption rates normalized to whole body lean mass. E: \(\text{VCO}_2\) production rates normalized to whole body lean mass. Data are presented as means ± SE for a 3-day measurement; \(n = 3–6\)/group. *\(P < 0.05\) vs. LFD mice (controls).
for 20 min. Tissue section was rinsed twice with TBST for 5 min, blocked in TBST (20 mM Tris, 137 mM NaCl, and 0.05% Tween-20, pH 7.5) containing 10% BSA at room temperature for 40 min, and incubated in TBST containing anti-CD68 antibody at room temperature for 2 h. Tissue slide was washed three times for 5 min with TBST. A horseradish peroxidase-conjugated secondary antibody (Bio-Rad) was used for staining at room temperature for 1 h. After being washed three times, metal enhanced 3,3-diaminobenzidine-tetrahydrochloride substrate was added to tissue and incubated for 15 min. Tissue was also counterstained with hematoxylin stain for 1 min, washed with ultrapure water, and mounted with mounting medium (Thermo Fisher Scientific, Waltham, MA) (18).

Local levels of cytokines (IL-6, TNFα, IL-1β, IFNγ, and IL-10) and chemokine [macrophage chemoattractant protein (MCP-1)] in white adipose tissue, skeletal muscle, liver, and heart, as well as serum cytokines (IL-1α, IL-5, IL-6, and IL-10), were measured using multiplexed Luminex. Skeletal muscle expression of glucose transporter 4 (GLUT4), AMP-activated protein kinase (AMPK) and phospho-AMPK (Thr172) were determined using quadriceps muscle and antibodies from Cell Signaling Technology.

Lipid measurement. Skeletal muscle (quadriceps) and liver triglyceride levels were measured by digesting tissue samples in chloroform-methanol and using a triglyceride assay kit (Sigma Diagnostics, St. Louis, MO).

Calculation. Rates of basal hepatic glucose production (HGP) and insulin-stimulated whole body glucose turnover were determined as described previously (13). Insulin-stimulated rate of HGP during the clamp was determined by subtracting the glucose infusion rate from whole body glucose uptake. Whole body glycolysis and glycogen plus lipid synthesis from glucose were calculated as described previously (13). Insulin-stimulated glucose uptake in individual tissues was assessed by determining the tissue (e.g., skeletal muscle, heart) content of 2-[14C]DG-6-P and plasma 2-[14C]DG profile.

Statistical analysis. Differences between groups were examined for statistical significance using the analysis of variance with Fisher’s test.

RESULTS

A short-term LFD and exercise intervention mediate a rapid weight loss in obese mice. After 12 wk of high-fat feeding, male C57BL/6 mice became obese, with more than a fourfold increase in whole body fat mass compared with age-matched, LFD-fed control mice (13.3 ± 0.7 g in HFD vs. 3.3 ± 0.3 g in LFD controls, P < 0.001). Whole body lean mass was modestly but significantly increased by 10% in HFD-fed mice (27.9 ± 0.5 vs. 25.4 ± 0.5 g in LFD controls, P < 0.005). Weight loss was mediated by switching to a LFD or adding a

![Figure 3](http://ajpendo.physiology.org/)

Fig. 3. Weight loss improves glucose homeostasis in obese mice. A 2-h hyperinsulinemic euglycemic clamp was performed to measure insulin sensitivity and glucose metabolism in awake mice (LFD, n = 7; HFD, n = 8; HFD → LFD, n = 8; HFD + Ex, n = 6). A: basal plasma glucose levels following overnight fast (~15 h). B: basal plasma insulin levels. C: plasma insulin levels during hyperinsulinemic euglycemic clamps. D: steady-state glucose infusion rates required to maintain euglycemia during clamps. E: time course of glucose infusion rates during hyperinsulinemic euglycemic clamps. Data are presented as means ± SE. *P < 0.05 vs. LFD mice.
cage wheel for exercise while continuing HFD in mice. Both interventions resulted in a steady decline in body weight over the course of 21 days, with LFD switch achieving a greater loss of body weight than exercise intervention (~17% weight reduction after LFD switch compared with respective baseline vs. ~13% reduction after exercise compared with respective baseline; Fig. 1A). Whole body fat mass was reduced by 45% following a switch to LFD (12.6 ± 1.3 vs. 6.9 ± 1.0 g after LFD switch; Fig. 1B). Whole body fat mass was also reduced by 30% after exercise (14.9 ± 0.9 vs. 10.3 ± 1.6 g after exercise). Despite dramatic reductions in adiposity, both groups of mice remained relatively obese compared with the control mice fed LFD (2.6 ± 0.2 g of fat mass; Fig. 1B). Whole body lean mass was not affected by a short-term weight loss intervention (Fig. 1C).

Changes in energy balance during weight loss in obese mice. To determine the effects of different weight loss interventions on energy balance, a 3-day metabolic cage study was conducted to measure daily food intake, physical activity, and energy expenditure using indirect calorimetry. Daily food intake, measured in kcal/day to account for different caloric energy expenditure using indirect calorimetry. Daily food intake was not significantly different in any of the groups except for the exercise group, which showed higher food intake compared with LFD mice (Fig. 2A). Thus, the short-term weight loss regimen in our study did not involve a caloric restriction or reduction in caloric intake in mice.

Energy expenditure rates were normalized to whole body lean mass, measured using 1H-MRS, because of a significant difference in body weight between HFD and weight loss intervention groups. Energy expenditure rates were elevated by ~20% in the exercise group compared with HFD mice, and this increase was observed during the day and night cycles, suggesting that such effects are not directly coupled to exercise or activity (Fig. 2, B and C). V̇O₂ consumption rate was significantly elevated in the exercise group, and V̇CO₂ production rates were elevated in both weight loss intervention groups compared with HFD mice (Fig. 2, D and E). Respiratory exchange ratios were comparable in both HFD-fed groups during the last week of the 12-wk initial period before intervention and was not significantly different in any of the groups [10.3 ± 1.4 kcal/day for LFD, 9.9 ± 0.7 kcal/day for HFD, 8.3 ± 1.9 kcal/day for HFD → LFD, and 8.8 ± 0.3 kcal/day for HFD + exercise (EX)]. Following 3-wk intervention, daily food intake was not significantly different in any of the groups except for the exercise group, which showed higher food intake compared with LFD mice (Fig. 2A). Thus, the short-term weight loss regimen in our study did not involve a caloric restriction or reduction in caloric intake in mice.

Fig. 4. Weight loss improves whole body insulin sensitivity in obese mice. A 2-h hyperinsulinemic euglycemic clamp was performed with [1H]glucose and 2-deoxy-D-[1-14C]glucose in awake mice to measure insulin sensitivity and glucose metabolism in individual organs (n = 6–8/group). A–C: insulin-stimulated whole body glucose turnover, glycolysis, and glycogen synthesis. D: insulin-stimulated glucose uptake in skeletal muscle (gastrocnemius). E: Insulin-stimulated Ser473 phosphorylation of Akt (ratio of phosphorylated Akt to total Akt levels) in skeletal muscle (gastrocnemius; n = 4–5/group). F: Intramuscular triglyceride levels (quadriceps; n = 4–6/group). Data are presented as means ± SE; n = 6–8/group unless otherwise noted. *P < 0.05 vs. LFD mice.

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Levels in C pAMPKα / AMPKα (0.86 ± 0.01 in the HFD group and 0.88 ± 0.01 in the HFD + EX group) but higher in LFD switch group (0.96 ± 0.01). In contrast, physical activity was not significantly different in any of the groups (80,603 ± 14,433 counts/day for LFD controls, 86,771 ± 17,435 counts/day for HFD, 120,251 ± 11,955 counts/day for HFD → LFD, and 88,650 ± 11,786 counts/day for HFD + EX; P > 0.05). To that end, physical activity (i.e., metabolic cage analysis) was measured for 3 days in the exercise group without running wheel. Thus, weight loss following exercise is due largely to increased energy expenditure in mice.

A short-term weight loss improves insulin sensitivity in obese mice. Plasma glucose and insulin levels were measured following overnight fast. HFD-induced obese mice developed fasting hyperglycemia, a hallmark of type 2 diabetes, and a LFD switch reduced plasma glucose levels to near-euglycemia (Fig. 3A). The exercise group remained hyperglycemic despite losing body weight. Basal plasma insulin levels were elevated more than threefold in HFD-fed mice compared with controls (LFD; Fig. 3B). Plasma insulin levels were significantly reduced following LFD-mediated weight loss and tended to be lower in the exercise group (Fig. 3B).

To determine the effects of weight loss on insulin sensitivity, we performed a 2-h hyperinsulinemic euglycemic clamp combined with [3H]glucose infusion in conscious mice. During the clamp, plasma glucose levels were maintained at ~7 mM, and plasma insulin levels were raised to approximately fourfold above respective basal levels in all groups of mice (Fig. 3C). Steady-state rates of glucose infusion required to maintain euglycemia during clamps were significantly reduced in HFD-fed mice, indicating diet-induced insulin resistance in this group (Fig. 3, D and E). Glucose infusion rates were significantly higher in both the LFD switch and exercise groups compared with HFD-fed mice (Fig. 3, D and E), suggesting improved insulin sensitivity in both groups of weight loss intervention.

Insulin-stimulated whole body glucose turnover rates were reduced after HFD, but a LFD switch or exercise almost normalized whole body glucose turnover in mice (Fig. 4A). Insulin-stimulated whole body glycolysis and glycogen synthesis rates were also reduced after HFD but normalized after a LFD switch or exercise (Fig. 4, B and C). Taken together, these data indicate that an ~15% weight loss following a short-term LFD switch or exercise caused a dramatic improvement in whole body insulin sensitivity in obese mice.

Improved glucose metabolism in individual organs is dependent on weight loss modality. Tissue-specific glucose uptake was measured using 2-[14C]DG injection during clamps. Insulin-stimulated glucose uptake in skeletal muscle was significantly reduced in HFD-fed mice compared with LFD mice (Fig. 4D). Skeletal muscle insulin signaling was examined using Western blot and showed that HFD-induced insulin resistance involved a 50% reduction in Ser473 phosphorylation of protein kinase B (Akt; Fig. 4E). Intramuscular triglyceride levels were elevated more than sevenfold after HFD, and this is likely responsible for muscle insulin resistance in HFD-fed mice (Fig. 4F) (2). Exercise-mediated weight loss markedly

![Fig. 5](http://ajpendo.physiology.org/) Exercise-mediated weight loss increases AMP-activated protein kinase (AMPK) protein levels in skeletal muscle. A: total AMPKα protein levels in skeletal muscle (quadriceps). B: phospho-AMPKα (p-AMPKα; Thr172) in skeletal muscle (quadriceps). C: ratio of p-AMPKα/total AMPKα protein levels in muscle. D: total glucose transporter 4 (GLUT4) protein levels in skeletal muscle (quadriceps). Data are presented as means ± SE; n = 3/group. *P < 0.05 vs. LFD mice.
improved muscle glucose metabolism, but skeletal muscle remained insulin resistant following a LFD intervention (Fig. 4D). Insulin-stimulated Akt serine phosphorylation remained lower in both weight loss groups, including exercise mice that showed normalized expression of muscle glucose uptake (Fig. 4E). Total Akt protein levels were not affected by HFD or weight loss intervention. Intramuscular triglyceride levels remained higher in all groups of mice compared with LFD-fed controls (Fig. 4F). To further explore the underlying mechanism by which exercise-mediated weight loss improved muscle insulin sensitivity, we measured AMPK and GLUT4 protein expression in skeletal muscle. Total AMPKα protein levels in skeletal muscle were significantly elevated in the exercise group compared with controls (Fig. 5A). Thr172 phosphorylation of AMPK and the phospho-AMPKα/AMPKα ratio in skeletal muscle tended to be higher in the exercise group (Fig. 5, B and C). Total GLUT4 protein levels were not significantly different in any of the groups (Fig. 5D). Thus, skeletal muscle glucose metabolism was improved following weight loss, but selectively in exercise group.

Basal HGP rates tended to rise after HFD (P = 0.05), consistent with fasting hyperglycemia in these mice (Fig. 6A). Basal HGP rates in the LFD switch or exercise group did not differ compared with LFD controls. Hepatic insulin action, expressed as insulin-mediated percent suppression of basal HGP, was dramatically reduced after HFD, indicating HFD-induced hepatic insulin resistance (Fig. 6B). Interestingly, hepatic insulin action was significantly improved in LFD switch mice (~30% increase compared with HFD-fed mice), whereas the exercise group remained insulin resistant in liver (Fig. 6B). Unaffected hepatic insulin resistance in the exercise group was consistent with higher basal glucose levels in these mice (Fig. 3A). Intrahepatic triglyceride levels were elevated almost threefold in HFD-fed mice compared with LFD-fed controls (Fig. 6C). Weight loss mediated by a LFD switch or exercise dramatically reduced intrahepatic lipid content to levels indistinguishable from LFD mice (Fig. 6C). Thus, HFD-induced fatty liver was ameliorated following LFD or exercise-mediated weight loss, but hepatic insulin action was selectively improved following a diet intervention.

Insulin-stimulated glucose uptake in white and brown adipose tissue was significantly reduced after HFD, and neither weight loss intervention had any beneficial effects on adipose tissue glucose metabolism (Fig. 6, D and E). In contrast, insulin-stimulated glucose uptake in the heart was reduced in HFD-fed mice, and HFD-mediated cardiac insulin resistance was significantly improved following LFD or exercise intervention (Fig. 6F).

Fig. 6. Weight loss alters glucose and lipid metabolism in individual organs. A: basal hepatic glucose production (HGP) in mice. B: hepatic insulin action expressed as insulin-mediated %suppression of HGP. C: intrahepatic triglyceride levels (n = 4–6/group). D and E: insulin-stimulated glucose uptake in white (epididymal) and brown (intrascapular) adipose tissue. F: insulin-stimulated glucose uptake in heart. Data are presented as means ± SE; n = 6–8/group unless otherwise noted. *P < 0.05 vs. LFD mice.
A short-term weight loss following LFD switch does not ameliorate adipose tissue inflammation in mice. Recent studies have implicated an important role of adipose tissue inflammation in obesity-mediated insulin resistance (32). Circulating levels of cytokines IL-1α, IL-5, and IL-10 were not significantly different between groups, although plasma IL-6 levels tended to be lower in both weight loss intervention groups compared with HFD mice (Fig. 7). Adipose tissue levels of inflammatory cytokines IL-1β, IL-6, TNFα, and IFNγ were all significantly elevated in HFD-fed mice (Fig. 8). Adipose expression of CD68 as a marker of local macrophage population and MCP-1 levels were also increased twofold in HFD-fed mice compared with LFD-fed controls (Fig. 8, A and E). HFD-induced adipose tissue inflammation was largely unaffected by LFD-mediated weight loss intervention, as adipose tissue levels of CD68, IL-1β, IL-6, MCP-1, and IFNγ remained higher compared with LFD controls. Exercise-mediated weight loss also did not affect adipose levels of IL-1β and IL-6 but tended to lower TNFα, MCP-1, and IFNγ levels. Adipose tissue IL-10 levels were significantly elevated after LFD switch (Fig. 8G). Thus, a short-term weight loss did not significantly attenuate adipose tissue inflammation in mice.

HFD-induced inflammation in liver, skeletal muscle, and heart is attenuated following weight loss. In addition to adipose tissue inflammation, local inflammation in other insulin-sensitive organs, such as liver and skeletal muscle, is involved in obesity-mediated insulin resistance (25). Local CD68 levels, as a marker of macrophage content, were measured using Western blot in liver samples obtained from mice. Liver CD68 levels were increased more than threefold in HFD-fed mice (Fig. 9A). Weight loss mediated by a LFD switch or exercise caused a significant reduction in liver CD68 levels. Local levels of proinflammatory cytokines IL-6 and TNFα were also elevated after HFD but reduced after weight loss intervention (Fig. 9, B and C).

Skeletal muscle expression of CD68 was also markedly elevated in HFD-fed mice, suggesting increased macrophage infiltration into skeletal muscle following HFD (Fig. 10A). Consistent with this notion, local levels of inflammatory cytokines TNFα and IL-6 were significantly increased in skeletal muscle after HFD (Fig. 10, B and C). Muscle TNFα and IL-6 levels were normalized after weight loss intervention. Muscle IL-1β levels were not affected by HFD or weight loss (Fig. 10D). Furthermore, local levels of MCP-1 and IFNγ in heart were significantly elevated in HFD-fed mice compared with LFD-fed controls (Fig. 11, A and B). Following weight loss, myocardial levels of MCP-1 and IFNγ returned to levels comparable with those in LFD controls (Fig. 11, A and B). Myocardial TNFα and IL-1β levels did not show an identifiable pattern in response to HFD or weight loss intervention (Fig. 11, C and D). Taken together, weight loss mediated by a LFD or exercise intervention resulted in a system-wide attenuation of HFD-induced inflammation in liver, skeletal muscle, and heart.

**DISCUSSION**

It is well established that modest weight loss provides significant beneficial effects on glucose homeostasis in obese and diabetic subjects. Although weight loss intervention pro-
vides a hopeful solution against a devastating global trend in obesity, it is important to understand the molecular basis of such effects. In this study, chronic high-fat feeding was used to induce obesity in male C57BL/6 mice, and dietary and exercise interventions were applied for 3 wk to mediate a short-term weight loss. Our findings confirm the beneficial effects of weight loss on systemic glucose homeostasis and inflammation, but importantly they indicate selective effects on insulin sensitivity that are dependent on the modality of weight loss.

After 12 wk of high-fat feeding, male C57BL/6 mice became obese, insulin resistant with fasting hyperinsulinemia, and diabetic with fasting hyperglycemia (~12 mM). Following 21 days of weight loss intervention, mice with a LFD switch from HFD showed a greater reduction in whole body adiposity than mice with free exercise using a cage wheel that remained on HFD. This may be due to the fact that exercise increased daily food consumption compared with other groups. The exercise effect on feeding behavior is a well-known phenomenon in humans, but there are inconsistent reports. Acute bouts of exercise have been shown to increase serum levels of satiety factors such as ghrelin and peptide YY and reduce food intake in human subjects (16). In contrast, a repeated exercise session for 3 days increased ad libitum food intake in obese subjects (8). These discordant findings may be due partly to acute vs. chronic effects of exercise on energy consumption (5). Furthermore, our data showed that a 3-wk exercise intervention

![Fig. 8. Short-term, modest weight loss does not significantly affect adipose tissue inflammation. Local levels of CD68, IL-1β, IL-6, TNFα, macrophage chemoattractant protein (MCP-1), IFNγ, and IL-10 in white adipose tissue (WAT; epididymal). Data are presented as means ± SE; n = 4–5/group. *P < 0.05 vs. LFD mice.](image)

![Liver CD68 Levels](image)

![Liver IL-6 Levels](image)

![Liver TNF-α Levels](image)

![Adipose Tissue CD68 Levels](image)

![IL-1β Levels in WAT](image)

![IL-6 Levels in WAT](image)

![TNF-α Levels in WAT](image)

![MCP-1 Levels in WAT](image)

![IFN-γ Levels in WAT](image)

![IL-10 Levels in WAT](image)

![Fig. 9. Short-term weight loss reduces local inflammation in liver. Local levels of CD68, IL-6, and TNFα in liver. Data are presented as means ± SE; n = 5/group. *P < 0.05 vs. LFD mice.](image)
increased energy expenditure rates, which were further attributed to a significant loss of adiposity in obese mice. In that regard, a recent study from Boström et al. (3) found that exercise stimulates skeletal muscle secretion of irisin, which was shown to increase energy expenditure in mice.

After 3 wk of LFD or exercise intervention, mice remained relatively obese with, two- to fourfold higher whole body fat mass than LFD-fed control mice. It is likely that mice may lose more obesity with a longer period of dietary or exercise intervention. Regardless, both LFD and exercise interventions were effective in improving glucose homeostasis. These data indicate that metabolic abnormalities induced by chronic high-fat feeding are reversible upon withdrawal of high-fat diet or exercise. This is consistent with a recent clinical study showing the beneficial effects of dietary intervention in insulin-resistant, overweight children (28). LFD intervention resulted in significant weight loss and improvement in glucose homeostasis, as shown by near-normalization of fasting glucose and insulin levels following a LFD switch from HFD. In contrast, exercise did not improve hepatic insulin resistance despite marked reversal of inflammatory response in liver. These data indicate that although hepatic inflammation may be responsible for HFD-induced insulin resistance in liver (27), other factors are involved in improved insulin sensitivity following weight loss mediated by a LFD or exercise. We have shown previously that ectopic lipid accumulation and intracellular increases in lipid-derived metabolites induce insulin resistance in liver (14). In that regard, we measured intrahepatic triglyceride levels and found that both modalities of weight loss completely normal-
ized intrahepatic lipid content. Thus, improved insulin sensitivity following weight loss mediated by a LFD may be attributed to significant reductions in local inflammation and lipid content in liver. On the other hand, exercise-mediated weight loss normalized local inflammatory state and lipid level in liver but failed to improve hepatic insulin action in mice. This may be due to the fact that intrahepatic triglyceride level is only a surrogate marker and may not necessarily reflect changes in intracellular lipid metabolism or metabolites in liver following exercise. Alternatively, our data suggest that factors other than lipid and inflammation may account for HFD-induced insulin resistance in liver.

Previously, we have shown that HFD-induced insulin resistance in skeletal muscle is due to local inflammation, and IL-10-mediated suppression of inflammation rescued muscle insulin resistance in obese mice (11). Obesity-mediated insulin resistance is also due to elevated levels of lipid-derived metabolites such as fatty acyl-CoAs and diacylglycerol and downregulation of insulin receptor substrate-associated insulin signaling in skeletal muscle (14, 15). In the current study, HFD-induced obesity increased local levels of CD68, IL-6, and TNFα and reduced glucose uptake in skeletal muscle. Intra-muscular triglyceride levels were also markedly elevated in HFD-fed mice, and these effects were associated with a significant decrease in Akt phosphorylation in muscle. Exercise-mediated weight loss dramatically attenuated local inflammation and increased glucose metabolism in skeletal muscle. Interestingly, muscle insulin signaling was not affected by exercise, and Akt phosphorylation remained impaired despite improved glucose metabolism in muscle. This is consistent with the fact that intramuscular lipid levels remained elevated after exercise. However, total AMPK protein levels were elevated in muscle following exercise. Furthermore, weight loss following a LFD switch reduced muscle inflammation but did not improve muscle glucose metabolism. Skeletal muscle insulin signaling and lipid levels were also minimally affected by LFD-mediated weight loss. These results indicate that exercise-specific effects possibly involving AMPK may be involved in the improvement of muscle glucose metabolism following exercise. Moreover, our recent study found activation of inflammatory response in the heart following HFD-induced obesity, and this was associated with cardiac insulin resistance (18). In the current study, high-fat feeding increased myocardial levels of MCP-1 and IFNγ, and weight loss following a LFD switch or exercise attenuated myocardial inflammation. Weight loss also improved myocardial glucose metabolism, supporting a cause-and-effect relationship between HFD-induced inflammation and insulin resistance in the heart.

Despite dramatic effects of short-term weight loss to reduce local inflammation in skeletal muscle, liver, and heart, adipose tissue inflammation was not ameliorated by a LFD or exercise. In fact, HFD-induced obesity caused a two- to threefold increase in adipose tissue levels of IL-1β, IL-6, and MCP-1, which were not significantly affected by weight loss. This is likely due to the fact that, although a 3-wk diet or exercise intervention decreased whole body adiposity, all of these mice remained relatively obese with 7–10 g of fat mass compared with less than 4 g of fat mass in lean control mice. These data support the notion that adipose tissue inflammation is directly related to whole body adiposity and suggest that further loss of
adiposity with longer intervention may reduce adipose tissue inflammation. Importantly, our findings indicate that suppression of skeletal muscle, liver, and heart inflammation following modest weight loss is sufficient to improve insulin sensitivity in these organs independent of adipose tissue inflammation. These results also suggest that HF-induced local inflammation in liver, skeletal muscle, and heart is not dependent on adipose tissue inflammation and may be associated with some unknown local factors. In that regard, Kosteli et al. (21) recently found that local lipid fluxes regulate adipose tissue inflammation. Further studies are needed to determine whether local lipid fluxes are also involved in liver and muscle inflammation in HF-fed mice.

In conclusion, our findings demonstrate that obesity-mediated inflammation plays a major role in insulin resistance in skeletal muscle, liver, heart, and adipose tissue. Short-term weight loss by diet or exercise intervention dramatically suppresses local inflammation in skeletal muscle, liver, and heart and improves insulin sensitivity in these organs without concomitant reduction in adipose tissue inflammation. Finally, exercise has a selective effect to increase skeletal muscle glucose metabolism, whereas a LFD intervention selectively improves hepatic insulin action in obese mice.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


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