Improved insulin sensitivity after weight loss and exercise training is mediated by a reduction in plasma fatty acid mobilization, not enhanced oxidative capacity

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Obesity is characterized by excessive rates of plasma fatty acid mobilization and uptake, which play a key role in mediating insulin resistance. While weight loss via diet-only or a diet + exercise program clearly improves insulin sensitivity, the precise mechanisms modulating this improvement are not completely understood. The purpose of the present study was to determine the role of the reduced fatty acid mobilization and uptake after weight loss in obese women who were randomly assigned to lifestyle interventions of either weight loss without exercise (WL) ($n = 7$) or a weight loss + exercise program (WL + EX) ($n = 10$). Before and after losing 12% of their body weight, we measured insulin sensitivity (S_I), systemic fatty acid rate of appearance (Ra) and disappearance (Rd), oxidative capacity, and markers for pro-inflammatory pathways in skeletal muscle. Fatty acid Ra and Rd were reduced by ~30% after both interventions ($P < 0.05$). While oxidative capacity increased 25% in WL + EX (compared with no increase after WL), the improvement in S_I was identical in both groups (~60%; $P < 0.05$), and skeletal muscle pro-inflammatory pathways were reduced ($P < 0.05$) similarly in both groups. When we artificially increased fatty acid mobilization after weight loss to pre-weight-loss levels via an overnight lipid infusion, the improvement in S_I was almost completely reversed. Importantly, WL + EX did not protect against this lipid-induced reversal in S_I despite a significant increase in resting whole-body fat oxidation and a marked increase in skeletal muscle oxidative capacity. In conclusion, reduced fatty acid mobilization and uptake appears to be a primary mediator of improved insulin sensitivity after weight loss. Moreover, enhancing fatty acid oxidative capacity via exercise training is not sufficient to prevent the insulin resistance caused by high fatty acid mobilization, such as that found in obesity.

Abbreviations BMI, body mass index; COX-I, NADH-ubiquinol oxidoreductase; CPT-I, carnitine palmitoyl transferase-I; DEXA, dual energy x-ray absorptiometry; FFM, fat-free mass; FM, fat mass; GC-FID, gas chromatography with flame ionization detection; GC-MS, gas chromatography-mass spectrometry; HOMA, homeostasis model assessment; IKK, inhibitor of kB kinase; IVGTT, intravenous glucose tolerance test; JNK, c-Jun N-terminal kinase; NFκB, nuclear factor κB; PGC-1, peroxisome proliferator-activated receptor-γ co-activator 1; Ra, rate of appearance in plasma; Rd, rate of disappearance from plasma; S_I, insulin sensitivity; TTR, tracer–tracee ratio; $\dot{V}_{CO_2}$, rate of carbon dioxide production; $\dot{V}_{O_2}$, rate of oxygen consumption; $\dot{V}_{O_2,peak}$, peak rate of oxygen consumption; WL, weight-loss intervention; WL + EX, weight loss + exercise training intervention.

Introduction

Insulin resistance is among the most pervasive metabolic complications associated with obesity, and is linked with the pathogenesis of chronic diseases, such as type 2 diabetes and cardiovascular disease (Facchini *et al.* 2001). Weight loss through caloric restriction is known to attenuate and even reverse insulin resistance (Assali *et al.* 2001; Toledo *et al.* 2008; Ross *et al.* 2000; Janssen *et al.* 2002), and adding an endurance exercise training program to a calorie restricted diet has been proposed to augment improvements in insulin sensitivity (Goodpaster *et al.* 2003; Toledo *et al.* 2007). However, differentiating the effects of exercise training

from the effects of weight loss is very difficult. As such, the precise mechanisms that mediate improvements in insulin sensitivity after a combined weight-loss–exercise training intervention are poorly defined.

Recently, a reduced capacity of skeletal muscle to oxidize fatty acids, as well as impairments in mitochondrial function have been implicated as important mediators of insulin resistance in obesity (for review, Savage *et al.* 2007), although some clinical studies do not support this perspective (Ostergard *et al.* 2006; Boushel *et al.* 2007; Nair *et al.* 2008). Fatty acid oxidative capacity has been found to be lower in skeletal muscle from some obese subjects compared with lean controls (Kim *et al.* 2000; Berggren *et al.* 2008), and it has been hypothesized that enhancing oxidative capacity via endurance exercise training may be responsible for much of the improvement in insulin sensitivity after an exercise training–weight-loss program (Goodpaster *et al.* 2003; Berggren *et al.* 2004, 2008; Toledo *et al.* 2007). Several reports, however, indicate that moderately obese individuals (i.e. BMI: 30–40 kg m^{-2}) do not a have an impaired ability to oxidize fatty acids, yet they are still insulin resistant (Hulver *et al.* 2003; Bonen *et al.* 2004; Bandyopadhyay *et al.* 2006). In addition, improvements in insulin sensitivity after weight loss in obesity appear to occur independently of changes in skeletal muscle fatty acid oxidation, oxidative capacity, mitochondrial function or mitochondrial size (Berggren *et al.* 2008; Toledo *et al.* 2008). Therefore, the impact of alterations in oxidative capacity on insulin sensitivity remains unclear.

Interpretation of data from studies examining the effect of an exercise training/weight-loss program on insulin sensitivity is further complicated because weight loss (i.e. a reduction in fat mass) can reduce systemic fatty acid mobilization (Klein *et al.* 1996; Lofgren *et al.* 2002), which itself can improve insulin sensitivity (Santomauro *et al.* 1999; Bajaj *et al.* 2005). In addition, studies by Ross and colleagues (Ross *et al.* 2000; Dekker *et al.* 2007) have demonstrated that exercise training, and a resultant increase in oxidative capacity, does not improve insulin sensitivity when subjects do not lose weight. This may be explained by the fact that exercise training without weight loss does not reduce fatty acid mobilization and uptake (Horowitz *et al.* 1999*a*, 2000). Moreover, when the degree of weight loss is similar, there does not appear to be an additional benefit of adding exercise training to a weight-loss program to the improvement in insulin sensitivity (Ross *et al.* 2000; Janssen *et al.* 2002), although the mechanism(s) for these comparable improvements in insulin sensitivity are unknown.

It has become increasingly evident that chronic, low-grade inflammatory responses play an important role in mediating insulin resistance in obesity (Schenk *et al.* 2008). For example, the activity of pro-inflammatory serine/stress kinase pathways, such as inhibitor of kB (IkB) kinase (IKK)–nuclear factor κB (NFκB), and c-Jun N-terminal kinase (JNK), are elevated in skeletal muscle in obesity and type 2 diabetes (Hirosumi *et al.* 2002; Bandyopadhyay *et al.* 2005; Ropelle *et al.* 2006; Sriwijitkamol *et al.* 2006). These pathways are important because they are activated by increased fatty acid availability and can negatively regulate insulin signalling (Nguyen *et al.* 2005). Therefore, these pro-inflammatory signals provide an intermediate molecular link between fatty acid metabolism and insulin action. Conversely, endurance training in obese, diabetic subjects suppresses the activation of the IKK–NFκB pathway, as evidenced by an increased abundance of IκB-α and IκB-β (Sriwijitkamol *et al.* 2006). Although a single session of exercise is known to reduce JNK activation (Ropelle *et al.* 2006; Schenk & Horowitz, 2007), the effect of exercise training on JNK activity is less clear. Considering the potential opposing effects of fatty acid mobilization and exercise on the activation of the IKK–NFκB and JNK pathways, it is possible that the addition of exercise to a weight-loss program may help improve insulin sensitivity at least in part through a reduced pro-inflammatory/stress response.

The primary aim of the present study was to investigate whether decreased fatty acid mobilization or an increase in skeletal muscle oxidative capacity is more important in mediating the improvement in insulin sensitivity in response to adding an endurance exercise training program to a weight-loss intervention. We also sought to gain a better understanding of the effects of weight loss (with and without exercise training) on the activation of the pro-inflammatory JNK and IKK–NFκB pathways.

Methods

Subjects

Seventeen abdominally obese women (body mass index (BMI): 30–40 kg m^{-2}; waist circumference >100 cm) participated in this study. All subjects were premenopausal and were considered to be in good health after a comprehensive medical examination, which included a history and physical examination, a 12-lead electro-cardiogram and standard blood and urine tests. No subject was taking regular medications (except birth control). All subjects were non-smokers, weight stable (±2 kg) for 4–6 months and had not exercised regularly for at least 6 months before beginning the study. Subjects with type 2 diabetes, coronary heart disease, clinically significant hypertriglyceridaemia (i.e. plasma triglycerides >150 mg dl^{-1}) or hypertension were excluded. Subject characteristics are presented in Table 1. Some of these subjects also participated in a previous study evaluating the affect of weight loss and exercise training on the regulation of intracellular fatty acid transport (Schenk & Horowitz, 2006). All subjects were fully informed of the possible

Table 1. Subject characteristics before and after weight loss

	Weight loss (WL)			Weight loss + Exercise (WL + EX)		
	Before	After	%Δ	Before	After	%Δ
Age (years)	30 ± 3	–	–	30 ± 2	–	–
Body weight (kg)	88.6 ± 2.9	78.0 ± 2.5*	−12 ± 0%	91.9 ± 2.8	80.9 ± 2.5*	−12 ± 0%
BMI (kg m^{-2})	34 ± 2	30 ± 2*	−12 ± 0%	33 ± 1	29 ± 1*	−12 ± 0%
% body fat	47.9 ± 1.5	44.1 ± 2.2*	−8 ± 2%	47.1 ± 0.8	42.9 ± 1.3*	−9 ± 2%
Fat mass (kg)	42.6 ± 2.4	34.6 ± 2.6*	−19 ± 2%	43.4 ± 1.6	34.7 ± 1.6*	−20 ± 2%
Fat-free mass (kg)	46.0 ± 1.1	43.4 ± 1.3*	−6 ± 2%	48.5 ± 1.5	46.1 ± 1.5*	−5 ± 2%
$V_{O_2,peak}$ (l min^{-1})	2.1 ± 0.1	2.0 ± 0.1	−3 ± 3%	2.1 ± 0.13	2.6 ± 0.1*	+22 ± 4%

Values are means ± S.E.M. *$P \leq 0.01$, compared with Before.

risks associated with the study and signed an informed consent document, which was approved by the University of Michigan Institutional Review Board, in accordance with the *Declaration of Helsinki*.

Preliminary testing

Whole-body fat mass (FM) and fat-free mass (FFM) were determined before and after weight loss using dual-energy x-ray absorptiometry (Lunar DPX DEXA Scanner, Madison, WI, USA). Aerobic fitness was assessed before and after weight loss by measuring peak oxygen uptake ($\dot{V}_{O_2,peak}$) during cycle ergometer exercise. The protocol consisted of a 4 min warm-up, followed by a progressive increase in work rate every minute until volitional fatigue.

Experimental procedures

An overview of the experimental design is presented in Fig. 1. More specifically, on three separate occasions (once before (Before) and twice after weight loss), subjects were admitted to the General Clinical Research Center (GCRC) at the University of Michigan hospital at 1800 h, and stayed overnight for a 'metabolic study'. After admission, an intravenous catheter was placed in a hand vein for periodic overnight blood sampling. During the two trials after weight loss, a second intravenous catheter was placed in a forearm vein for either an overnight infusion of saline (After) or lipid plus heparin (After + Lipid). The goal of the lipid-plus-heparin infusion was to increase fatty acid mobilization back up to levels similar to that found before weight loss, which enabled us to assess the metabolic impact of the lower fatty acid mobilization resulting from their weight loss. During the lipid infusion, subjects were infused overnight with a 20% lipid emulsion (0.2 ml kg^{-1} min^{-1}; Abbott Laboratories, North Chicago, IL, USA) and heparin (5 U kg^{-1} min^{-1}; Elkins-Sinn, Inc., Cherry Hill, NJ, USA). Our preliminary data indicated that this infusion rate would return plasma fatty acid mobilization to levels similar to those found before weight loss. A standardized meal (15 kcal kg^{-1} FFM) was provided

at 2000 h and subjects remained fasted until completion of the trial the next day.

At 0700 h the next morning, a muscle biopsy was obtained from the vastus lateralis muscle. Muscle samples were dissected free of adipose and connective tissue, rinsed in saline, blotted dry, frozen in liquid nitrogen, and stored at −80°C until analysis, as described below. After the muscle biopsy procedure, another intravenous catheter was placed in a forearm vein for isotope infusion. At 0800 h, we began a 1 h constant infusion of [L-^{13}C]palmitate (0.04 μmol kg^{-1} min^{-1}) (Cambridge Isotope Laboratories, Andover, MA, USA) bound to human albumin (Baxter, Deerfield, IL, USA). After 45 min of the isotope infusion (time necessary to achieve isotopic equilibrium in the body (Sidossis *et al.* 1995)), four arterialized blood samples were collected in 5 min intervals from a heated hand vein for determination of fatty acid Ra and Rd (i.e. fatty acid mobilization and uptake). The rates of oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$) were also measured for 20–30 min intervals at 0730 h and again at 0830 h, using a metabolic cart (Delta Trac, Sensor Medics Yorba Linda, CA, USA) to calculate the rate of fasting whole-body fatty acid oxidation. After these measurements of fatty acid metabolism, an intravenous glucose tolerance test (IVGTT) was conducted to assess insulin sensitivity (S_I) using the minimal model technique, as previously described (Schenk *et al.* 2005). After the IVGTT, subjects were fed and after vital signs were stable they were discharged from the hospital. All trials were conducted during the first 2 weeks of the subjects' menstrual cycle. The two trials after weight loss were separated by at least 7 days, and for the exercise training group both trials were conducted exactly 3 days after the subjects' exercise session.

Weight-loss interventions

The weight-loss intervention used in this study has been previously described in detail (Schenk & Horowitz, 2006). Briefly, after completing the baseline metabolic

study, subjects were instructed to reduce their caloric intake 500–800 kcal day^{-1} below that required to maintain body weight, with the goal to achieve a rate of weight loss of 0.5–1.0 kg week^{-1}. Subjects met weekly with our research dietitian. After losing 12% of their initial body weight, dietary caloric intake was increased in order to meet the energy needs of weight maintenance, and subjects were in weight maintenance (±0.5 kg) for at least 3–4 weeks before being admitted to the hospital for their follow-up metabolic studies. Subjects in the weight-loss treatment group (WL, $n = 7$) were not permitted to exercise or increase their physical activity level. For subjects in the weight-loss plus exercise treatment group (WL + EX, $n = 10$), in addition to the aforementioned dietary program, these subjects also performed supervised exercise on a stationary bicycle ergometer for 3 days per week for 45 min at 85% of maximum heart rate, with one additional unsupervised session per week. Exercise training continued throughout the weight maintenance period. After weight loss and the weight maintenance period, the first follow-up metabolic study was completed exactly 3 days after exercise. Between the first and second post-weight-loss trials, all WL + EX subjects performed two to three exercise sessions, and again there was exactly 3 days between the last exercise session and the second metabolic study.

Analytical methods

Plasma substrate and hormone levels. Plasma glucose (Thermo DMA, Melbourne, Australia) and overnight fatty acids (Waco Chemicals GmbH, Neuss, Germany) concentrations were measured by colorimetric assay. For samples taken during the tracer infusion, plasma palmitate and total plasma fatty acid concentration were measured by internal standard method using gas chromatography with flame ionization detection (GC/FID) (Wolfe, 1992). Plasma insulin concentration was measured by radio-immunoassay (Linco Research, Inc., St Louis, MO, USA).

Plasma fatty acid kinetics. The tracer-to-tracee ratio (TTR) for plasma palmitate was determined by gas chromatography-mass spectrometry (GC-MS) with an MSD 5973 system (Agilent Technologies; Wilmington, DE, USA) with capillary column as previously described (Patterson *et al.* 1998).

Western blotting. Whole-cell lysates were prepared as described previously (Schenk & Horowitz, 2006). For Western blotting, samples (25 μg) were separated by 8% SDS-PAGE and transferred in an ice bath at 200 mA. After blocking, membranes were probed for phosphorylated-JNK (p-JNK; no. 9251, Cell Signaling, Danvers MA, USA), JNK-1 (sc-474; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and IκB-β (nos 9242 and 9248; Cell Signaling Technology). Membranes were then incubated in the appropriate secondary antibody for 60 min and were developed with enhanced chemiluminescence (Amersham Biosciences, Piscataway, NJ, USA). Bands were quantified by densitometry (Fluor Chem SP, Alpha Innotech, San Leandro, CA, USA). As all samples were not run on the same gel, or on the same
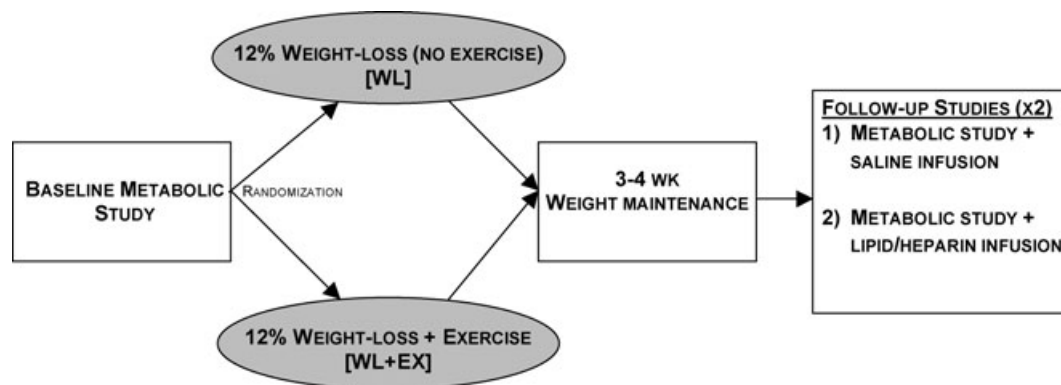
Figure 1. Overall experimental design
Subjects were admitted to the hospital for a battery of baseline metabolic tests to assess fatty acid metabolism and insulin sensitivity (see details in Experimental procedures). Subjects were then randomized into one of two weight-loss treatment groups: (1) weight loss through dietary intervention only (WL), or (2) weight loss through an intervention of diet and exercise training (WL + EX) (see details in Weight-loss interventions). Subjects followed their respective weight-loss interventions until they lost 12% of their initial body weight, at which point they were placed on a weight-maintaining diet for 3–4 weeks before being admitted to the hospital again for their follow-up studies. Subjects completed two separate follow-up metabolic studies. On one of these occasions subjects were tested identically as they were before the intervention, with a constant saline infusion used as a control. On the other occasion subjects received a low-dose lipid and heparin infusion throughout their hospital visit (see details in Experimental procedures). These follow-up studies were separated by at least 1 week.

day, and to control for differences in film exposure time, band densities for each subject on each blot were expressed relative to a standard (muscle from an obese female), which was run in duplicate on all gels.

Calculations

Resting whole-body fatty acid oxidation was calculated from resting $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ measurements using the equations of Frayn (Frayn, 1983). Palmitate rate of appearance (Ra) and disappearance (Rd) were calculated using the Steele equation for steady-state conditions (Steele, 1959). Fatty acid Ra was calculated by dividing palmitate Ra by the ratio of plasma palmitate to total plasma fatty acid concentration, as measured by GC/FID. As measurements were made under steady-state conditions, fatty acid Ra = fatty acid Rd. The insulin sensitivity index (S_I) was calculated from least-squares fitting of the insulin and glucose concentration curves from the IVGTT using the Minimal Model Millennium (version 6.02; MinMod Inc.) computer analysis software. It must be noted that S_I does not allow us to differentiate insulin sensitivity among different tissues. However, S_I has been found to closely match insulin-stimulated glucose disposal into skeletal muscle as measured by the hyperinsulinaemic–euglycaemic clamp technique (Bergman *et al.* 1987; Saad *et al.* 1994). Therefore, changes in S_I are probably reflective of changes in skeletal muscle insulin sensitivity.

Statistics

A two-way ANOVA for repeated measures with Tukey *post hoc* analysis was used to assess differences among treatments for all parameters. Statistical significance was set at $P < 0.05$. All values are presented as mean ± S.E.M.

Results

### Changes in body weight and body composition

There were no differences in age, initial body weight or body composition between the groups (Table 1). As designed, all subjects in the WL and WL + EX groups lost 12% of their initial body weight (Table 1). Overall, the time taken to reach the goal body weight was significantly shorter in WL + EX compared with WL (20 ± 2 *vs.* 30 ± 3 weeks, $P < 0.01$). All subjects maintained their lower body weight for 4 weeks before the follow-up metabolic studies. BMI, % body fat and fat mass (FM) also declined significantly ($P < 0.01$), with no differences between groups. Both weight-loss treatments also slightly, yet significantly ($P < 0.01$), reduced fat-free mass (FFM). Exercise training significantly increased whole-body oxidative capacity, as shown by a $22 \pm 4\%$ increase in $\dot{V}_{O_2,peak}$ in the WL + EX group (Table 1, $P < 0.0001$). In the WL group, however, oxidative capacity was unchanged (Table 1).

Plasma triglyceride concentration

Plasma triglyceride concentration was not affected by weight loss in either the WL or WL + EX groups (Fig. 2). Moreover, the lipid infusion after weight loss did not significantly increase plasma triglyceride concentration (Fig. 2), indicating that nearly all of the infused triglycerides were hydrolyzed.

Fatty acid mobilization, uptake and oxidation

Plasma fatty acid concentration measured throughout the overnight hospital visit was not significantly altered by either of the two weight-loss treatments (Fig. 3A). Despite the similar plasma fatty acid concentration before and after weight loss, fatty acid mobilization and uptake (fatty acid Ra and Rd) were more than 30% lower ($P < 0.03$) after weight loss in both groups (Fig. 3B). Importantly, during After + Lipid, fatty acid Ra and Rd
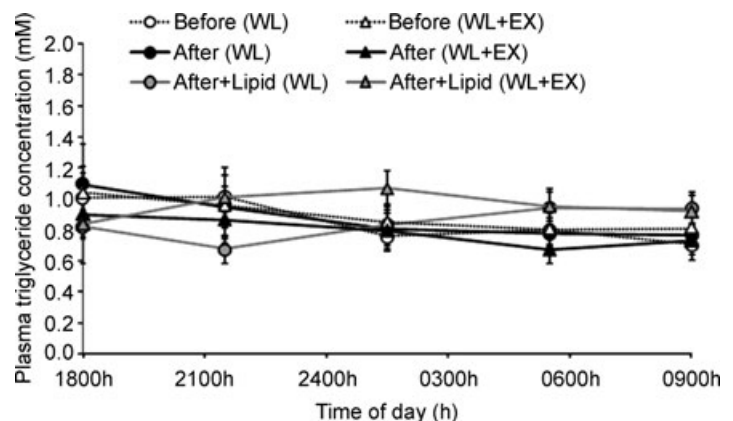
Figure 2. Plasma triglyceride concentration during the overnight hospital visits
Open circles, before WL; black circles, after WL; grey circles, after + Lipid WL; open triangles, before WL + EX; black triangles, after WL + EX; grey triangles, after + Lipid WL + EX.
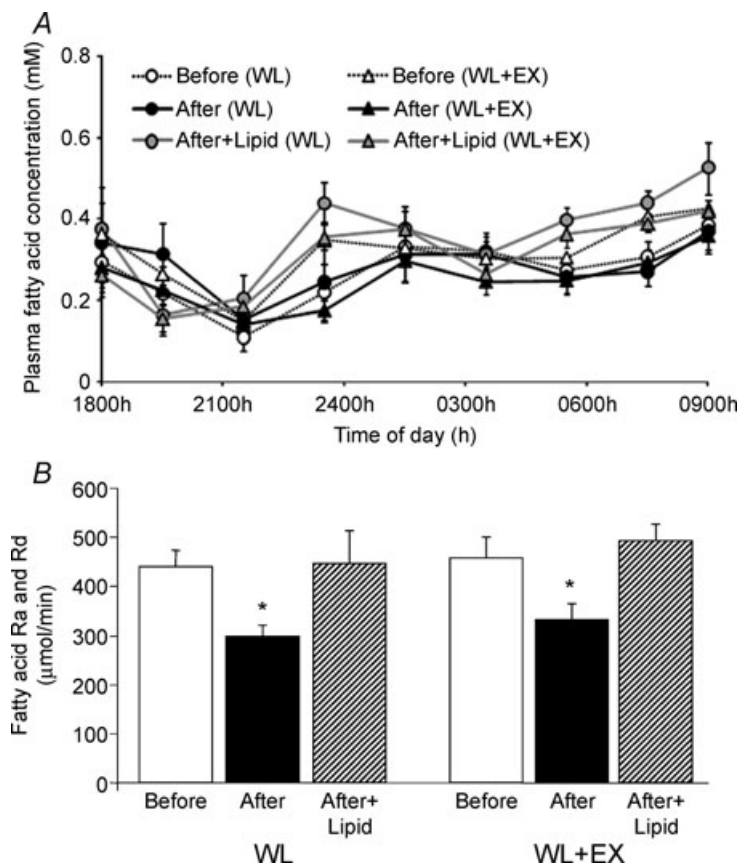
Figure 3

*A*, plasma fatty acid concentration during the overnight hospital visits. Open circles, before WL; black circles, after WL; grey circles, after + Lipid WL; open triangles, before WL + EX; black triangles, after WL + EX; grey triangles, after + Lipid WL + EX. *B*, fatty acid rate of appearance in plasma (Ra) and disappearance from plasma (Rd) after an overnight fast. Measurements were made before (Before) and after (After) weight loss, and during the lipid–heparin infusion after weight loss (After + Lipid). *Significantly different from Before and After + Lipid, $P < 0.05$.

were increased back to pre-weight-loss levels (Fig. 3*B*), and plasma fatty acid concentration was not different from levels observed before and after weight loss (Fig. 3*A*). Despite the reduction in fatty acid Rd (a measure of fatty acid uptake), resting fatty acid oxidation was unchanged after the intervention in WL. Consistent with an increase in maximal oxidative capacity, resting whole-body fatty acid oxidation was increased more than 20% after WL + EX (Fig. 4). This increase was due to an increase in the contribution of fatty acids to total energy expenditure (i.e. decreased respiratory exchange ratio; $P < 0.05$), because

resting energy expenditure was unchanged in WL and WL + EX (data not shown). Increasing fatty acid Ra and Rd after weight loss during After + Lipid did not further increase whole-body fatty acid oxidation in either group (Fig. 4). Notably, fatty acid Ra per kilogram FM was similar before and after weight loss (WL: 2.3 ± 0.1 *vs.* 2.1 ± 0.2 μmol (kg FM)$^{-1}$ min^{-1}, WL + EX: 2.5 ± 0.2 *vs.* 2.4 ± 0.2 μmol (kg FM)$^{-1}$ min^{-1}, Before *vs.* After), which suggests that the decrease in fatty acid mobilization after weight loss was due to a decrease in total fat mass.
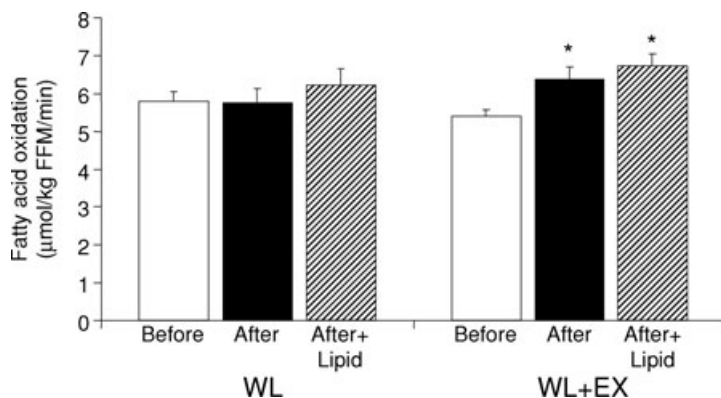
Figure 4. Whole-body fatty acid oxidation before (Before) and after (After) subjects lost 12% of their initial body weight, and during a lipid–heparin infusion after weight loss (After + Lipid)
*Significantly different from Before, $P < 0.05$.

Table 2. Plasma glucose and insulin concentrations

		Before	After	After + Lipid
Glucose (mM)	WL	5.0 ± 0.2	5.0 ± 0.2	5.0 ± 0.2
	WL + EX	5.1 ± 0.1	5.0 ± 0.1	4.9 ± 0.2
Insulin (μU ml^{-1})	WL	14.1 ± 1.8	9.3 ± 1.0*	10.2 ± 1.3*
	WL + EX	14.8 ± 1.4	10.6 ± 1.4*	11.9 ± 1.4*

Values are means $\pm$ S.E.M.

Fasting plasma glucose and insulin concentrations, and insulin sensitivity

Fasting plasma glucose concentrations were not affected by either weight-loss treatment (Table 2). However, fasting plasma insulin concentration declined 30–40% ($P < 0.05$) after weight loss in both groups (Table 2). Consistent with this reduction in fasting plasma insulin concentration, S_I increased by 60–70% ($P < 0.05$) after both treatments (Fig. 5), and the improvement in S_I was identical between treatments. Importantly, the lipid infusion and the resultant increase in fatty acid Ra and Rd completely reversed the improvement in S_I after weight loss in both groups (Fig. 5).

### Skeletal muscle pro-inflammatory factors

Total JNK protein abundance was not affected by either weight-loss intervention, or the lipid infusion. However, p-JNK (expressed relative to total JNK abundance) was reduced by ~40% after weight loss in both groups(Fig. 6A). The lipid infusion increased p-JNK back to levels found before weight loss in the WL group (Fig. 6A). Interestingly, although there was a trend for the lipid infusion after weight loss in WL + EX to increase p-JNK levels, this did not reach statistical significance ($P = 0.08$), and it remained significantly lower than Before ($P < 0.05$; Fig. 6A). Protein abundance of IκB-β was significantly increased by ~50% after both WL and WL + EX ($P < 0.05$; Fig. 6B). Importantly, the lipid infusion reduced IκB-β abundance back to pre-weight-loss levels in both treatment groups.

Discussion

Lifestyle interventions involving weight loss and exercise clearly improve insulin sensitivity in obese individuals, yet the mechanisms for this effect are not well understood. One of the major findings of this study was that the reduction in systemic fatty acid mobilization and uptake after weight loss plays a primary role in the insulin sensitizing effects of a weight-loss intervention. In contrast to evidence suggesting that alterations in fatty acid oxidative capacity may be an important contributor to the regulation of insulin sensitivity (Savage et al. 2007), our findings demonstrate that a marked elevation in oxidative capacity after WL + EX did not improve insulin sensitivity any more than WL. Moreover, we found that improved fatty acid oxidative capacity after exercise training was not sufficient to prevent insulin resistance when fatty acid flux was returned to pre-weight-loss levels with the lipid infusion during After + Lipid. Our findings also indicate that the improved insulin sensitivity after weight loss may in part be due to a reduction in the pro-inflammatory/stress response in skeletal muscle.

The excessive fatty acid mobilization, such as that found in persons with abdominal obesity (Horowitz et al. 1999b), is known to potently impair insulin sensitivity (Griffin et al. 1999; Roden et al. 2000; Itani et al. 2002). Here we demonstrated that a 30% reduction in fatty acid Rd
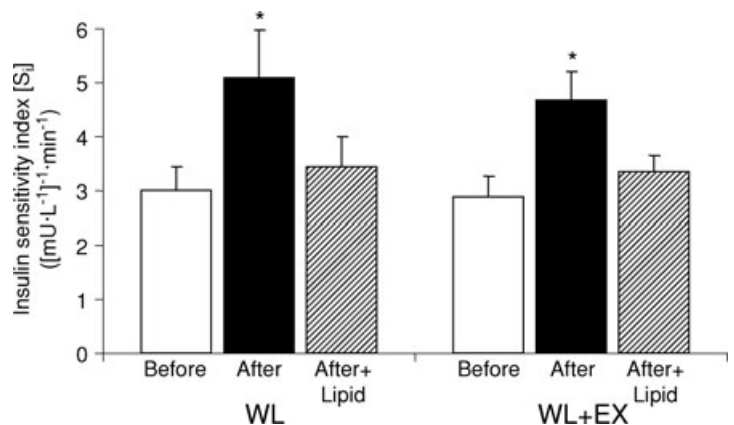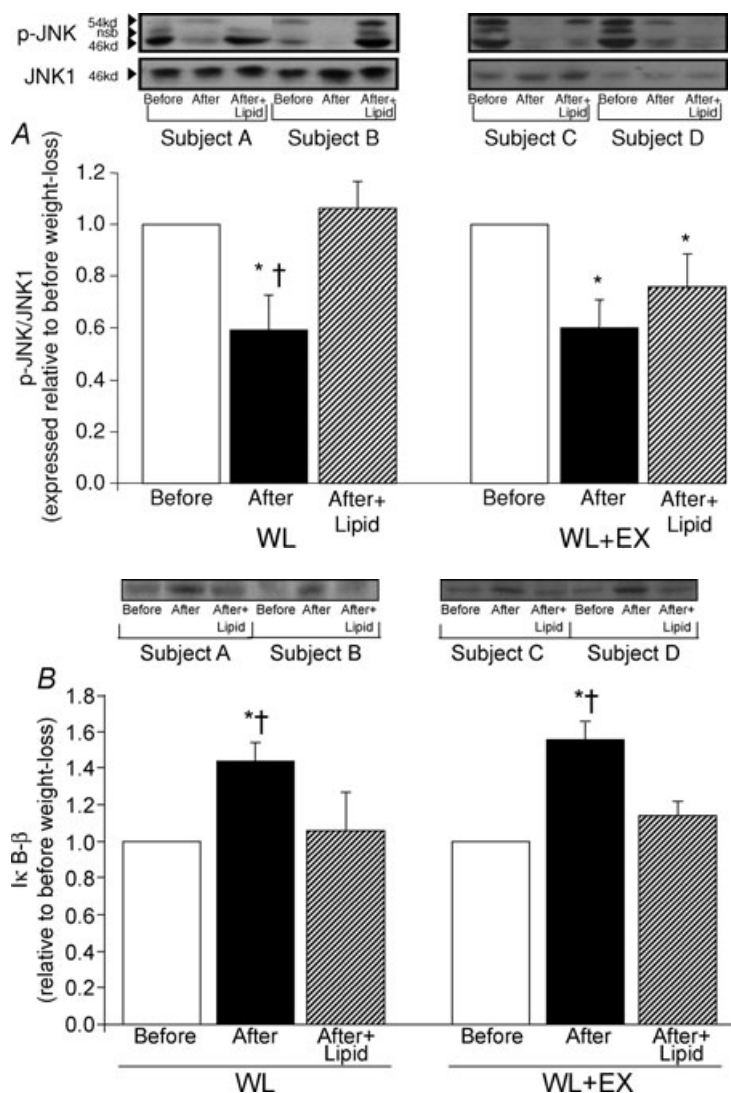

Figure 5. Insulin sensitivity index (S_I) before (Before) and after (After) subjects lost 12% of their initial body weight, and during a lipid–heparin infusion after weight loss (After + Lipid)
*Significantly different from Before and After + Lipid, $P < 0.05$.

(i.e. fatty acid uptake) after weight loss, regardless of whether or not the participant increased their aerobic fitness through exercise training, was accompanied by a > 60% improvement in insulin sensitivity. This is in line with studies in which insulin sensitivity was significantly enhanced after fatty acid mobilization was reduced by pharmacological inhibition of lipolysis (Santomauro *et al.* 1999; Bajaj *et al.* 2005). Our novel finding that the improvement in insulin sensitivity was effectively reversed when fatty acid Ra and Rd were returned to levels found before the intervention (via a lipid and heparin infusion; After + Lipid) indicates that the primary mediator of the improvement in insulin sensitivity after weight loss was a reduction in fatty acid mobilization. Mechanistically, we believe that the decrease in fatty acid mobilization after weight loss was simply due to the ~8 kg decrease in total fat mass, as fatty acid Ra per kilogram of FM, was unchanged after weight loss. It has previously been found that weight loss is accompanied by a ~40% reduction in

adipose tissue hormone-sensitive lipase abundance (Klein *et al.* 1996), which may also help explain the suppression in fatty acid mobilization after weight loss.

Interestingly, when weight loss is not accompanied by a reduction in fatty acid mobilization, insulin sensitivity is not improved (Klein *et al.* 2004). A study by Klein *et al.* (2004) reported that large-scale liposuction that resulted in the removal of a substantial amount of body fat (i.e. ~10 kg of body fat) did not reduce fatty acid mobilization, and insulin sensitivity was not improved. The reason why the liposuction treatment did not reduce fatty acid mobilization is not clear, but may be due to the removal of fat cells with the liposuction procedure, which subsequently may reduce the ability of the body to sequester fatty acids. Other studies using conventional weight-loss programs (i.e. caloric restriction without surgery) have also reported that fatty acid mobilization and/or lipolytic rate is not decreased by weight loss, or may even be elevated (Kanaley *et al.* 1993; Vazquez & Kazi, 1994). In these

Figure 6

A, abundance of phosphorylated c-Jun NH_2-terminal kinase (p-JNK) expressed relative to total JNK-1 abundance (arbitrary units) and *B*, protein abundance of inhibitor κB ($I\kappa$B)-β in muscle biopsy samples obtained before (Before) and after (After) subjects lost 12% of their initial body weight, and during a lipid–heparin infusion after weight loss (After + Lipid). Inset, Western blots of p-JNK, total JNK and $I\kappa$B-β abundance from two representative subjects. *Significantly different from Before. †Significantly different from After + Lipid, $P < 0.05$. nsb, non-specific binding.

studies, however, lipolytic rate or fatty acid mobilization were measured while the subjects were still in a negative energy balance, which is a time when the hormonal milieu (e.g. elevated catecholamine and suppressed insulin concentrations) is conducive for an elevated lipolytic rate. Therefore, measuring fatty acid mobilization when subjects are still in a negative energy balance does not accurately reflect the impact of the weight loss, *per se.* Studies measuring fatty acid mobilization during a period of weight stability after weight loss agree with our findings that weight loss results in a marked reduction in fatty acid mobilization, along with a concomitant improvement in generalized markers for insulin sensitivity (i.e. fasting insulin and/or homeostasis model assessment (HOMA)) (Klein *et al.* 1996; Lofgren *et al.* 2002; Thyfault *et al.* 2004).

Similar to findings from Ross and colleagues (Ross *et al.* 2000; Janssen *et al.* 2002) and more recently Toledo *et al.* (Toledo *et al.* 2008), we found that adding exercise training to a weight-loss program did not improve insulin sensitivity any more than weight loss without exercise training. Our observation that exercise training did not prevent fatty acid-induced insulin resistance in After + Lipid, is also consistent with a cross-sectional study that demonstrated that a lipid infusion impaired insulin sensitivity to a similar extent in both endurance exercise-trained and sedentary individuals (Matzinger *et al.* 2002). Importantly, the observation that exercise training does not augment insulin sensitivity appears to be contingent on removing the transient effects of the most recent session of exercise (Ivy *et al.* 1983; Mikines *et al.* 1989). In line with this, the subjects in our WL + EX group did not perform exercise for 3 days before the follow-up tests, and subjects in the studies by Ross and colleagues (Ross *et al.* 2000; Janssen *et al.* 2002; Dekker *et al.* 2007) and Toledo *et al.* (Toledo *et al.* 2008) were tested at least 3 days after their last exercise session. Our subjects, however, clearly responded to the exercise training intervention as demonstrated by their significant increase in whole-body aerobic capacity (i.e. $\dot{V}_{O_2,max}$), increased protein abundance of mitochondrial proteins (e.g. COX-I and CPT-I) in skeletal muscle (reported previously: Schenk & Horowitz, 2006), as well as increased resting fatty acid oxidation. Nevertheless, this enhancement in fatty acid oxidative capacity in WL + EX did not translate into an additive improvement in insulin sensitivity compared with WL, nor did it appear to be the mediator of improved insulin sensitivity after WL + EX. That is, because our lipid infusion reversed insulin sensitivity similarly in WL and WL + EX, our data suggest that the reduction in fatty acid mobilization after weight loss is the key determinant for the weight-loss-induced increase in insulin sensitivity regardless of whether the participants had exercise-trained (and increased their fatty acid oxidative capacity) or not.

The impact of oxidative capacity and fatty acid oxidation on the regulation of insulin sensitivity is controversial. Although previous reports indicate that insulin resistant offspring of type 2 diabetics have low oxidative capacity (Petersen *et al.* 2004), improved insulin action after exercise training in offspring of type 2 diabetics was found to be dissociated from an increase in oxidative capacity (Ostergard *et al.* 2006). Additionally, in contrast to our finding that improved oxidative capacity did not protect against fatty acid-induced insulin resistance, over-expression of CPT-I, and overexpression of peroxisome proliferator-activated receptor-γ co-activator 1 (PGC-1), have been found to prevent fatty acid-induced insulin resistance *in vitro* (Sinha *et al.* 2004; Koves *et al.* 2005), as well as *in vivo* (Bruce *et al.* 2009). However, it is unclear whether findings from these *in vitro* and animal studies translate well to *in vivo* human physiology. For example, similar to our findings, a higher capacity to oxidize fat in lean endurance-trained subjects did not protect against lipid-induced insulin resistance (Matzinger *et al.* 2002). Similarly, non-diabetic Asian Indians have been found to be highly insulin resistant when compared to northern Europeans controls, despite their skeletal muscle having a greater capacity for oxidative phosphorylation (Nair *et al.* 2008). Perhaps most relevant to our present findings, it is important to recognize that even when endurance training is found to induce a significant increase in resting fatty acid oxidation *in vivo* (as in the present study), the magnitude of this increase is typically very small (i.e. 10–20 μmol min^{-1}), especially when compared with the rate of fatty acid mobilization in persons with abdominal obesity (i.e. 400–600 μmol min^{-1}). Accordingly, even if fatty acid oxidative capacity is elevated, this is probably insufficient to compensate for the excessive fatty acid mobilization and uptake found in obesity. This perspective is further supported by recent studies demonstrating that although a high-fat diet in rodents increased their rates of fatty acid oxidation and skeletal muscle fatty acid oxidative capacity, insulin sensitivity was still reduced because this increase in fat oxidation (and oxidative capacity) was insufficient to accommodate the excess fatty acid flux (Turner *et al.* 2007; Hancock *et al.* 2008; Bruce *et al.* 2009).

To gain a better understanding of the mechanisms by which weight loss improved insulin sensitivity in our study we measured the activation of pro-inflammatory JNK and IKK–NFκB pathways in skeletal muscle. The activation of these pathways is elevated in skeletal muscle from obese individuals and type 2 diabetics (Bandyopadhyay *et al.* 2005; Sriwijitkamol *et al.* 2006). Fatty acids have been found to activate the JNK and IKK–NFκB pathways, and increased fatty acid mobilization and obesity appears to cause insulin resistance in skeletal muscle, at least in part, via activation of these pathways (Hirosumi *et al.* 2002; Ropelle *et al.* 2006). To this end, we found that weight loss, the resultant reduction in fatty acid mobilization and improvement in insulin sensitivity were accompanied

by reduced p-JNK and increased IκB-β abundance (i.e. suggestive of a reduced activation of the IKK–NFκB pathway), and these changes occurred independently of exercise training. To our knowledge, this is the first study to demonstrate that weight loss reduces inflammation in human skeletal muscle. The comparable reductions in activation of the JNK and IKK–NFκB pathways may also help explain the similar improvement in insulin sensitivity between our two treatment groups. Further support for a role of these pathways in the improvement in insulin sensitivity after weight loss was demonstrated by our observation that the improvement in insulin sensitivity after weight loss was completely reversed during the lipid infusion trials, in conjunction with a reduction in IκB-β abundance and an elevation in p-JNK. Interestingly, our finding that the increase in p-JNK with lipid infusion during WL + EX did not quite reach statistical significance ($P = 0.08$) suggests that exercise training and/or improved oxidative capacity, may have provided some protection against the susceptibility of JNK activation to increase in response to an excessive fatty acid mobilization. Nevertheless, this attenuation did not prevent fatty acid-induced insulin resistance.

Despite the vast number of studies that have examined the effects of exercise training on insulin sensitivity, this topic remains controversial. We believe that much of this controversy can be resolved by comparing the experimental designs and the specific research questions being addressed in studies in this area. Among the greatest confounding issues in these studies is body weight loss that often accompanies exercise/lifestyle interventions. Since a negative energy balance clearly evokes a potent increase in insulin sensitivity (Assali *et al.* 2001), it is not surprising that an exercise training program would enhance insulin sensitivity when the participant is studied in a state of negative energy balance (Goodpaster *et al.* 2003). To accurately assess the effects of exercise training independently of the effects of a negative energy balance it is critical to study the participants after a period of weight stability. In addition, because the degree of weight loss can also affect the magnitude of metabolic adaptations, when attempting to assess the impact of adding an exercise training regimen to a dietary/lifestyle intervention, it is imperative to match the weight lost by subjects in the different interventions. Finally, because the effects of a single session of exercise can persist for 2–3 days after exercise (Mikines *et al.* 1988; Cartee *et al.* 1989), to evaluate the effects of exercise training (and increased oxidative capacity), *per se*, on insulin sensitivity (and independently of the acute effects of exercise) metabolic measurements should not be performed for at least ∼3 days after the most recent session of exercise, and diets after the most recent exercise session should be controlled, at least in terms of providing enough dietary carbohydrate to fully replenish glycogen stores. When all of these issues are accounted for,

there is a general consensus in the literature that much of the effect of exercise training on enhancing insulin sensitivity is primarily due to the residual effects of the most recent exercise session, and that increasing oxidative capacity via exercise training is not a potent enhancer of insulin sensitivity (Ivy *et al.* 1983; Mikines *et al.* 1989; Perseghin *et al.* 1996; Janssen *et al.* 2002; Ross *et al.* 2004; Dekker *et al.* 2007; Toledo *et al.* 2008). Nevertheless, it is very important to note that exercise training, which represents the accumulation of numerous acute bouts of exercise, certainly provides many benefits to metabolic and overall health.

An important limitation of the present study was that our measurement of insulin sensitivity (i.e. IVGTT) measures whole-body insulin sensitivity, and does not provide a direct measurement of skeletal muscle insulin sensitivity. Notably, however, approximately 85% of measured and calculated S_I from the IVGTT has been shown to be the result of insulin increasing glucose uptake into skeletal muscle (Bergman *et al.* 1987). Additionally, fatty acid-induced insulin resistance has been found to be primarily due to defects in skeletal muscle insulin action (Dresner *et al.* 1999; Itani *et al.* 2002). Nevertheless, undoubtedly weight loss would result in improvements in insulin sensitivity in all major metabolic tissues, including liver, adipose tissue and skeletal muscle. Another limitation of the present study was that because the Liposyn solution we infused during the After + Lipid trials was over 60% linoleate, and only ∼10% palmitate, the accuracy of our fatty acid Ra/Rd measurement during the lipid infusion trials requires that palmitate kinetics resemble the kinetics for linoleate and the other major fatty acid species. Importantly, it has been found that fractional uptake of palmitate is similar to many of the major fatty acid species, including linoleate (Hagenfeldt *et al.* 1972), and palmitate and linoleate are both considered to provide a reasonable estimate of total fatty acid kinetics (Mittendorfer *et al.* 2003). Moreover, it has been reported that there were no differences in the metabolism of the major individual fatty acid species from a lipid emulsion solution similar to that provided in our study (Fielding *et al.* 1999). Additionally, our infusion rate of the exogenous 20% lipid solution ($0.2 \, \text{ml kg}^{-1} \, \text{h}^{-1}$) would theoretically yield an increase in fatty acid Ra of ∼200 μmol min^{-1}, if all fatty acids were liberated from the infused triglycerides (TG) ($0.2 \, \text{ml kg}^{-1} \, \text{h}^{-1} \times 0.2 \, \text{g TG ml}^{-1} \times 1 \, \text{g TG} \, (880 \, \text{mol})^{-1} \times 3 \, \text{mol fatty acid} \, (1 \, \text{mol triglyceride})^{-1} \times 90 \, \text{kg} \times 1 \, \text{h} \, (60 \, \text{min})^{-1} = 204 \, \mu\text{mol min}^{-1}$). This theoretical increase in exogenous fatty acid Ra matched very well with our measured increase in fatty acids Ra between After and After + Lipid (150–200 μmol min^{-1}). This provides further evidence that our tracer methods did indeed provide an accurate reflection of whole-body fatty acid Ra/Rd.

In summary, enhanced insulin sensitivity after weight loss (regardless of whether weight loss occurred via dietary restriction alone or dietary restriction combined with exercise training) was primarily mediated by a reduction in fatty acid mobilization. In addition, although adding exercise training to a dietary weight-loss intervention increased fatty acid oxidative capacity and resting fatty acid oxidation, these changes were not sufficient to prevent fatty acid-induced insulin resistance. Finally, the reduction in fatty acid mobilization and uptake after weight loss was associated with reduced activation of the pro-inflammatory JNK and IKK–NFκB pathways in skeletal muscle, and thus may be an important contributor to the weight loss-induced improvement in insulin sensitivity.

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Author contributions

This study was conducted in the Michigan Clinical Research Center within the University of Michigan Hospital, and in the Substrate Metabolism Laboratory in the School of Kinesiology at the University of Michigan. S.S., M.P.H., C.S., J.F.H. and C.F.B. were involved in study design and data collection, and data interpretation. S.S., M.P.H. and J.F.H. were involved in data analysis. All authors contributed to the drafting/revising of this paper, and approved of the final submitted version.

Acknowledgements