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## Effects of exercise training and diet on lipid kinetics during free fatty acid-induced insulin resistance in older obese humans with impaired glucose tolerance

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<sup>1</sup>Department of Pathobiology, Lerner Research Institute; <sup>2</sup>Department of Gastroenterology/Hepatology, Cleveland Clinic, Cleveland, Ohio; <sup>3</sup>Division of Cardiology, University of Maryland School of Medicine, Baltimore, Maryland; <sup>4</sup>Department of Nutrition, Case Western Reserve School of Medicine; and <sup>5</sup>Department of Physiology, Case Western Reserve University, Cleveland, Ohio

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**Solomon TP, Haus JM, Marchetti CM, Stanley WC, Kirwan JP.** Effects of exercise training and diet on lipid kinetics during free fatty acid-induced insulin resistance in older obese humans with impaired glucose tolerance. *Am J Physiol Endocrinol Metab* 297: E552–E559, 2009. First published June 16, 2009; doi:10.1152/ajpendo.00220.2009.—Elevated free fatty acids (FFA) are implicated with insulin resistance at the cellular level. However, the contribution of whole body lipid kinetics to FFA-induced insulin resistance is not well understood, and the effect of exercise and diet on this metabolic defect is not known. We investigated the effect of 12 wk of exercise training with and without caloric restriction on FFA turnover and oxidation (FFA<sub>ox</sub>) during acute FFA-induced insulin resistance. Sixteen obese subjects with impaired glucose tolerance were randomized to either a hypocaloric ( $n = 8$ ;  $-598 \pm 125$  kcal/day,  $66 \pm 1$  yr,  $32.8 \pm 1.8$  kg/m<sup>2</sup>) or a eucaloric ( $n = 8$ ;  $67 \pm 2$  yr,  $35.3 \pm 2.1$  kg/m<sup>2</sup>) diet and aerobic exercise (1 h/day at 65% of maximal oxygen uptake) regimen. Lipid kinetics ([1-<sup>14</sup>C]palmitate) were assessed throughout a 7-h,  $40 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$  hyperinsulinemic euglycemic clamp, during which insulin resistance was induced in the last 5 h by a sustained elevation in plasma FFA (intralipid/heparin infusion). Despite greater weight loss in the hypocaloric group ( $-7.7 \pm 0.5$  vs.  $-3.3 \pm 0.7\%$ ,  $P < 0.001$ ), FFA-induced peripheral insulin resistance was improved equally in both groups. However, circulating FFA concentrations ( $2,123 \pm 261$  vs.  $1,764 \pm 194$   $\mu\text{mol/l}$ ,  $P < 0.05$ ) and FFA turnover ( $3.20 \pm 0.58$  vs.  $2.19 \pm 0.58$   $\mu\text{mol} \cdot \text{kg FFM}^{-1} \cdot \text{min}^{-1}$ ,  $P < 0.01$ ) during hyperlipemia were suppressed only in the hypocaloric group. In contrast, whole body FFA<sub>ox</sub> was improved in both groups at rest and during hyperlipemia. These changes were driven by increases in intracellular lipid-derived FFA<sub>ox</sub> ( $12.3 \pm 7.7$  and  $14.7 \pm 7.8\%$ ,  $P < 0.05$ ). We conclude that the exercise-induced improvement in FFA-induced insulin resistance is independent of the magnitude of weight loss and FFA turnover, yet it is linked to increased intracellular FFA utilization.

lipid-induced insulin resistance; palmitate turnover; palmitate oxidation; diabetes; obesity; aging; physical activity

THE INHIBITORY EFFECT OF FREE FATTY ACIDS (FFA) on glucose metabolism was first proposed by Randle et al. (37) in 1964. Subsequent experiments led to the hypothesis that elevated FFA concentrations may be responsible for the manifestation of insulin resistance in type 2 diabetes (5, 18, 36). More recent work in lean healthy individuals has demonstrated that elevation of plasma FFA levels using lipid-heparin infusions has a

lipotoxic effect, impairing rates of whole body glucose disposal during hyperinsulinemic euglycemic clamps (13, 25, 28, 38, 49). Furthermore, experiments employing stable and radioisotope palmitate tracers show that, in healthy individuals, insulin suppresses FFA turnover and oxidation; yet during hyperinsulinemic hyperlipemic conditions, insulin is unable to suppress lipid metabolism (6, 31). Such effects have not been evaluated in clinically relevant obese, insulin-resistant groups.

Evidence also suggests that, compared with their lean counterparts, obese subjects have elevated basal FFA turnover, impaired basal FFA oxidation, and an attenuated insulin suppression of FFA turnover and oxidation, thus raising circulating FFA concentrations (8, 17). Therefore, obese individuals are described to be metabolically inflexible to the actions of insulin (27), and the underlying insulin resistance is likely due to lipotoxicity (34). Understanding the mechanism by which this phenomenon occurs is important due to the valuable outcome of improving insulin resistance in individuals at risk of developing diabetes and related macrovascular complications. An older, obese, impaired glucose-tolerant group is at great risk of such disease onset. Reducing plasma FFA concentrations using pharmaceutical agents (e.g., acipimox) has been shown to improve insulin sensitivity in healthy and diabetic individuals (33, 44). Furthermore, it has been demonstrated that weight loss and aerobic exercise have direct metabolic effects, improving not only insulin-stimulated glucose disposal but also whole body lipid oxidation (30, 32, 42). More specifically, exercise alone has been shown to elevate basal rates of lipolysis and lipid turnover in healthy individuals (15, 21), yet the effects of diet and exercise-induced weight loss in groups at high risk of developing diabetes-related complications are not fully understood. Certainly, hyperglycemia and hyperlipemia are key factors that contribute to disease onset; thus improving glycemic and lipemic control is a common therapeutic goal, particularly in older, obese, insulin-resistant populations. Here, we seek to investigate the effects of stimuli that are known to reduce insulin resistance on lipid kinetics (FFA turnover and oxidation) during basal, hyperinsulinemic, and hyperlipemic conditions. We hypothesized that additional weight loss via increases in physical activity and hypocaloric dietary intake would improve peripheral tissue insulin resistance more so than elevations in physical activity alone and that such changes would be driven by alterations in FFA availability and utilization.

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## EXPERIMENTAL PROCEDURES

**Subject recruitment.** Sixteen obese, weight-stable volunteers (3 male and 13 female, age  $66 \pm 1$  yr, body mass index  $34.0 \pm 1.4$  kg/m<sup>2</sup>) were recruited into our ongoing studies investigating the role of lifestyle intervention in insulin resistance. Following medical screening, which included a 12-lead electrocardiogram, an oral glucose tolerance test, and a full blood profile, volunteers were randomized to one of two exercise training study arms: a eucaloric group (EX) or a hypocaloric group (EX-HYPO). Only obese volunteers who exhibited impaired glucose tolerance [2-h plasma glucose 140–199 mg/dl (2)] from oral glucose tolerance test screening were eligible to partake in the study. Volunteers were screened out if they demonstrated any evidence of renal, hepatic, cardiovascular, or hematological disease or if they exhibited normal glucose tolerance. None of the subjects were taking pharmaceutical agents known to interfere with our outcome variables. Contraindication to increased levels of activity was also screened via an exercise stress test. All volunteers provided their written informed consent to participate in the study, and all procedures were approved by the Institutional Review Board at MetroHealth Medical Center.

**Intervention.** All subjects entered a lifestyle intervention involving 12 wk of aerobic exercise training and dietary counseling. The exercise component consisted of 60 min of exercise, 5 days/wk, performed at ~65% of the individual's maximal oxygen uptake ( $\dot{V}O_{2max}$ ) as dictated by a maximal exercise test. All exercise was fully supervised by an exercise physiologist; therefore, compliance was strictly monitored. These procedures are similar to those described in previous publications from our group (32, 42, 48). On the basis of indirect calorimetry measurements obtained during exercise training, we have determined that older obese subjects expend 400–500 kcal/h during these training bouts. The dietary component of the study was monitored via daily food consumption questionnaires plus weekly meetings with a registered dietician. Subjects randomized to the EX group were instructed to continue with their typical diet; subjects randomized to the EX-HYPO group were advised to reduce their typical caloric intake by ~500 kcal/day to induce a negative energy balance and facilitate additional weight loss. Caloric requirements were estimated using the Harris-Benedict equation, multiplied by a sedentary ( $\times 1.3$ ) physical activity correction factor (19). Subjects' "typical" diets were thoroughly analyzed prior to the study by using dietary record questionnaires. Prior to and immediately following the intervention, metabolic testing was performed in a controlled 3-day inpatient setting in the General Clinical Research Center.

**Participant characteristics.** Measurements of anthropometrics, whole body fat percentage (hydrostatic weighing), and maximal aerobic capacity during exhaustive exercise ( $\dot{V}O_{2max}$ ) were determined according to standardized procedures. The details of these particular protocols may be viewed in Solomon et al. (42).

**Insulin sensitivity and lipid kinetics.** Following an overnight (~10 h) fast, a hyperinsulinemic euglycemic clamp was performed in combination with an intralipid/heparin infusion and a primed continuous infusion of [1-<sup>14</sup>C]palmitate. A retrograde catheter was inserted into a dorsal hand vein and warmed (~60°C) in a heated box for arterialized venous sampling. An infusion site was identified in the antecubital fossa of the contralateral arm, and a second catheter was inserted into a median vein. A radiolabeled FFA infusate was prepared by complexing [1-<sup>14</sup>C]palmitate with human serum albumin to a final concentration of 10 mg/ml albumin, 0.5  $\mu$ Ci/ml [1-<sup>14</sup>C]palmitate, and 2.3 mg/ml sodium palmitate (47). At  $t = -90$  min, a primed (2.5  $\mu$ Ci) continuous (0.1  $\mu$ Ci/min) infusion of [1-<sup>14</sup>C]palmitate was begun and proceeded until the end of the clamp. Simultaneously, a bolus (3.5  $\mu$ Ci) of NaH<sup>14</sup>CO<sub>3</sub> was injected to prime the bicarbonate pool. At  $t = 0$  min, a hyperinsulinemic euglycemic clamp began as described previously (3, 10, 42). A primed 40 mU·m<sup>2</sup>·min<sup>-1</sup> infusion of insulin was started, and a variable rate 20% glucose infusion was used to titrate euglycemia (90 mg/dl). Glucose infusion rates were determined

using the calculations of DeFronzo et al. (10) on the basis of arterialized glucose concentrations measured every 5 min throughout the procedure. At  $t = 120$  min, to elevate plasma FFA concentrations, a continuous infusion of 20% intralipid (1.5 ml/min) and heparin (200 IU prime, then 15 IU/min) commenced, whereas hyperinsulinemia and euglycemia were maintained. This proceeded until the end of the clamp procedure at  $t = 420$  min. Blood samples were collected at 10-min intervals during the last 30 min of the baseline (basal:  $t = -30$  to 0 min), insulin-stimulated (INS; hyperinsulinemic stage:  $t = 90$ –120 min), and FFA-elevated periods (INS + FFA; hyperlipemic stage:  $t = 390$ –420 min) for the measurement of plasma FFA- and [1-<sup>14</sup>C]palmitate specific activity. Mean space-corrected glucose disposal rates (GDR) were also calculated during the last 30 min of the INS and INS + FFA periods. The clamped glucose concentrations for the hyperinsulinemic and hyperlipemic periods pre- and poststudy were as follows:  $90.4 \pm 0.8$ ,  $88.9 \pm 1.2$ ,  $89.6 \pm 0.5$ , and  $89.2 \pm 0.6$  mg/dl. The glucose variation coefficients during these same periods were  $4.1 \pm 0.5$ ,  $3.2 \pm 0.5$ ,  $2.7 \pm 0.3$ , and  $2.8 \pm 0.3\%$ . In addition, measurements of whole body substrate metabolism were estimated from indirect calorimetry through a ventilated hood. Expired air samples were analyzed via Hartmann-Braun (Frankfurt, Germany), differential paramagnetic O<sub>2</sub> (Magnos 4G), and nondispersive infrared CO<sub>2</sub> (Uras 4) analyzers (46). Samples were collected over a 30-min period at baseline and during the final 30 min of the INS and INS + FFA stages. At each of these stages, an expired air sample was also collected for analysis of <sup>14</sup>CO<sub>2</sub> specific activity.

**Analytical methods.** Plasma insulin (Millipore, Billerica, MA) and leptin (Linco Research, St. Charles, MO) were assayed using commercially available radioimmunoassays. Plasma glucose concentrations were measured using an automated glucose oxidase method (Beckman Analyzer II). Triglycerides (TG) and total cholesterol were analyzed on an automated platform (Roche Modular Diagnostics, Indianapolis, IN). Plasma FFA levels were determined using an enzymatic colorimetric procedure (NEFA C kit; Wako Chemicals, Dallas TX). Plasma [1-<sup>14</sup>C]palmitate was extracted from plasma (1 ml) using heptane-isopropanol (4 ml; 3:7), and specific activity was measured by liquid scintillation counting, as described previously (9, 11, 35). Expired air <sup>14</sup>CO<sub>2</sub> specific activity was also measured as described previously (17).

**Calculations.** Whole body lipid metabolism was calculated using the calculations of Frayn (14). Urinary nitrogen excretion rates were measured, and therefore, rates of carbohydrate and lipid oxidation were corrected for protein metabolism. Whole body lipid oxidation rates were divided by the molecular mass of a typical TG (palmitoyl-stearoyl-oleoyl-glycerol; M<sub>r</sub> = 861 g/mol) and multiplied by three (the number of FFAs in a TG) to derive a rate of whole body FFA oxidation (net FFA<sub>ox</sub>). FFA turnover was determined by dividing the [1-<sup>14</sup>C]palmitate infusion rate by the plasma [1-<sup>14</sup>C]palmitate specific activity (6, 35). The <sup>14</sup>CO<sub>2</sub> production rate was calculated as the product of the <sup>14</sup>CO<sub>2</sub> specific activity (disintegrations·min<sup>-1</sup>·mmol<sup>-1</sup>) and the CO<sub>2</sub> excretion rate from indirect calorimetry (mmol/min) divided by 0.81 (which represents the correction factor for the amount of labeled CO<sub>2</sub> not recovered from the bicarbonate pool in humans) (24, 35). Plasma lipid-derived FFA oxidation rate (plasma FFA<sub>ox</sub>) was then determined by dividing the <sup>14</sup>CO<sub>2</sub> production rate by the specific activity of plasma [1-<sup>14</sup>C]palmitate (17, 35). Nonplasma lipid-derived FFA oxidation (i.e., intracellular lipid derived and plasma TG derived) was estimated as the difference between plasma FFA<sub>ox</sub> and net FFA<sub>ox</sub> (6, 17, 35). Due to the well-established effect of exercise training to elevate intramyocellular lipid utilization (see DISCUSSION), the changes in nonplasma lipid-derived FFA oxidation should reflect changes in intramuscular lipid oxidation. Rates of lipid kinetics ( $\mu$ mol/min) are expressed per unit of fat-free mass (FFM).

**Statistical analyses.** A three-way (group  $\times$  study time point  $\times$  clamp time point) repeated-measures analysis of variance (ANOVA) was used to examine changes in lipid kinetics following the intervention. Two-way (group  $\times$  study time point) ANOVA was used to

Table 1. Subject characteristics for the 16 volunteers who underwent a 12-wk exercise training intervention in combination with either a eucaloric or a hypocaloric diet

Subject Characteristics (n = 16)	EX (2 M, 6 F)		EX-HYPO (1 M, 7 F)	
	Prestudy	Poststudy	Prestudy	Poststudy
Age, yr	67 ± 2		66 ± 1	
Weight, kg	96.0 ± 6.1	92.8 ± 6.0 <sup>c</sup>	88.4 ± 4.6	81.7 ± 4.4 <sup>c**</sup>
BMI, kg/m <sup>2</sup>	35.3 ± 2.1	34.0 ± 1.9 <sup>c</sup>	32.8 ± 1.8	30.2 ± 1.7 <sup>c**</sup>
FM, kg	42.2 ± 3.3	40.3 ± 3.5 <sup>b</sup>	36.0 ± 3.7	30.9 ± 3.7 <sup>b*</sup>
FFM, kg	54.4 ± 3.7	52.5 ± 3.2 <sup>a</sup>	52.4 ± 1.5	50.7 ± 2.0 <sup>a</sup>
FPG, mmol/l	6.11 ± 0.27	6.03 ± 0.25	6.03 ± 0.31	5.78 ± 0.14
FPI, μU/ml	24.9 ± 6.2	18.3 ± 3.9 <sup>b</sup>	16.3 ± 1.3	12.9 ± 1.3 <sup>b</sup>
Leptin, ng/ml	25.8 ± 5.6	23.3 ± 5.0 <sup>b</sup>	18.5 ± 4.8	13.6 ± 3.0 <sup>b</sup>
TG, mg/dl	196.8 ± 44.7	167.9 ± 25.4 <sup>a</sup>	209.3 ± 25.4	170.7 ± 27.5 <sup>a</sup>
Chol, mg/dl	209.4 ± 9.5	203.0 ± 10.4 <sup>b</sup>	222.9 ± 12.3	194.7 ± 10.6 <sup>c**</sup>
VO <sub>2max</sub> , l/min	1.83 ± 0.12	1.99 ± 0.13 <sup>a</sup>	1.80 ± 0.16	1.90 ± 0.11 <sup>a</sup>

Data represent means ± SE. Older obese men and women (n = 16) underwent 12 wk of exercise training with [eucaloric group (EX-HYPO)] or without [hypocaloric group (EX)] caloric restriction. M, males; F, females; BMI, body mass index; FM, whole body fat mass; FFM, whole body fat-free mass; FPG, fasting plasma glucose; FPI, fasting plasma insulin; TG, fasting triglycerides; Chol, fasting total cholesterol; VO<sub>2max</sub>, maximal oxygen consumption during exhaustive exercise. Baseline characteristics were not different between groups for any variable. <sup>a,b,c</sup>Statistical differences between pre- and postintervention means are identified, representing the 5, 1, and 0.1% levels of significance. \* and \*\*Group × study time point ANOVA interactions, indicating significance at the 5 and 1% levels.

analyze subject characteristics. In the event of a significant ANOVA *F* ratio, Bonferroni post hoc tests were applied to identify specific differences between means. Statistical significance was achieved if *P* < 0.05. All data are expressed as means ± SE.

## RESULTS

**Participant characteristics.** Table 1 describes the research volunteers' baseline (prestudy) characteristics. There were no differences between the EX or EX-HYPO groups for any variable at baseline (all comparisons, *P* > 0.05). The intervention-induced changes in body composition, metabolic status, and physical fitness variables are also highlighted in Table 1. Both groups demonstrated improvements in body weight and fat mass (FM). The changes were modest in the EX group (−3.3 ± 0.7% reduced body weight and −4.6 ± 2.4% reduced FM), whereas the effect was much greater in the EX-HYPO group (−7.7 ± 0.5% weight, −14.9 ± 4.1% FM, *P* < 0.01 vs. eucaloric). There were large, statistically significant decreases

in fasting insulin, leptin, TG, and total cholesterol; however, changes in fasting plasma glucose were not significant. VO<sub>2max</sub> was similarly improved in both groups (all subjects: +8.6 ± 2.9%; effect of time, *P* < 0.05). In addition, during the study the EX group consumed the same daily caloric load as in their habitual diet (1,658 ± 96 vs. 1,684 ± 196 kcal/day, *P* > 0.05), whereas the EX-HYPO group reduced their caloric intake by −598 ± 125 kcal/day (1,891 ± 148 vs. 1,293 ± 63 kcal/day, *P* < 0.05). Prestudy diets did not differ in macronutrient composition between groups; however, during the intervention, the EX-HYPO group ingested a lower percentage of their caloric load from fat (22.1 ± 1.8 vs. 32.4 ± 3.1% kcal, *P* < 0.05).

**Insulin sensitivity.** Figure 1 shows that insulin-stimulated GDRs (mg·kg FFM<sup>−1</sup>·min<sup>−1</sup>) were improved in both the EX (30.7 ± 12.2%) and EX-HYPO (31.5 ± 23.7%; effect of time: both *P* < 0.05) arms of the study. Prior to the intervention, intralipid/heparin infusion significantly reduced the insulin-

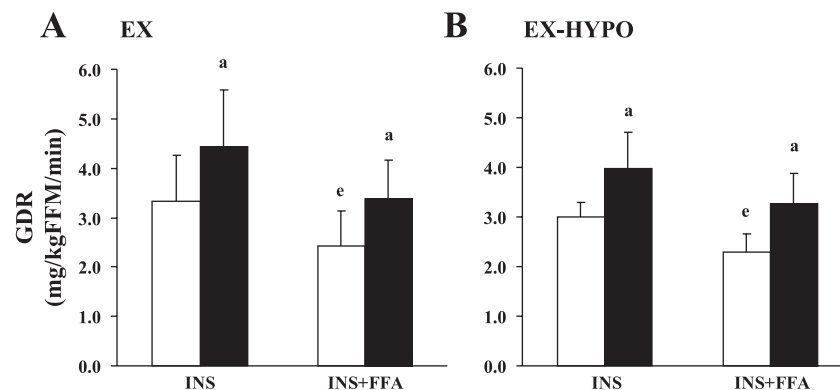


Fig. 1. Changes in glucose disposal rate (GDR) during the hyperinsulinemic hyperlipemic euglycemic clamp following a 12-wk exercise/diet intervention. Older obese men and women (n = 16) underwent 12 wk of exercise training (1 h/day, 5 days/wk at 65% maximal oxygen uptake). Participants either maintained their habitual dietary intake [eucaloric group (EX): 1,684 ± 196 kcal/day; A] or were counseled to reduce caloric intake [hypocaloric group (EX-HYPO): 1,293 ± 63 kcal/day; B]. Error bars represent SE. Open bars indicate preintervention means; filled bars represent postintervention means. The x-axis indicates the insulin-stimulated (INS) and intralipid/heparin (INS + FFA) stages of the clamp. Postintervention GDR was significantly elevated above baseline during both INS and INS + FFA stages in both groups (<sup>a</sup>pre- vs. postintervention, *P* < 0.05). Prior to the intervention, lipid infusion reduced GDR compared with insulin stimulation alone in both groups (<sup>e</sup>INS + FFA vs. INS, *P* < 0.05). Lipid-induced insulin resistance was no longer evident following the study (INS + FFA vs. INS, *P* = 0.17). FFM, fat-free mass.

stimulated GDR in both groups ( $P < 0.05$ ). Following the study, GDR during the INS + FFA stage was increased but notably was no longer significantly different from the INS stage ( $P = 0.17$ ). Improvements in insulin-stimulated GDR and FFA-induced insulin resistance were similar between groups (EX vs. EX-HYPO changes, group  $\times$  study time,  $P > 0.05$ ).

**Lipid kinetics.** Steady-state profiles of plasma FFA, breath  $^{14}\text{CO}_2$ , and plasma  $[1-^{14}\text{C}]$ palmitate specific activity are shown in Fig. 2. Changes in circulating FFA concentrations ([FFA]) are shown in Table 2. Both prior to and following the study, insulin infusion alone suppressed [FFA] ( $P < 0.05$ ), whereas [FFA] were elevated during the INS + FFA stage of the clamp ( $P < 0.05$ ). However, in the EX-HYPO group, postintervention [FFA] during basal and INS + FFA stages were reduced compared with prestudy values ( $P < 0.05$ ). Figure 3 illustrates changes in FFA turnover during basal, insulin-stimulated, and insulin hyperlipemic conditions. FFA turnover was effectively

suppressed during INS in both groups pre- and poststudy ( $P < 0.01$ ) but was elevated during the INS + FFA clamp stage ( $P < 0.01$ ). Following the hypocaloric intervention, FFA turnover was significantly reduced during basal and INS + FFA conditions (EX-HYPO,  $P < 0.01$ ; Fig. 3B); no change was seen in the EX group ( $P > 0.05$ ). Net  $\text{FFA}_{\text{ox}}$  was increased following the intervention during basal and INS + FFA conditions in both the EX and EX-HYPO groups ( $P < 0.05$ ; Table 2). Hyperinsulinemia effectively suppressed net  $\text{FFA}_{\text{ox}}$  in both groups, but only following the study (INS vs. basal: EX  $P < 0.05$ , EX-HYPO  $P < 0.01$ ). Also, following both interventions, during the hyperlipemic clamp stages, net  $\text{FFA}_{\text{ox}}$  was significantly elevated above rates measured during hyperinsulinemic conditions (INS + FFA vs. INS: EX  $P < 0.01$ , EX-HYPO  $P < 0.001$ ). No pre- vs. poststudy changes were identified in plasma lipid-derived  $\text{FFA}_{\text{ox}}$ , whereas significant increases in non-plasma lipid-derived  $\text{FFA}_{\text{ox}}$  during basal and INS + FFA stages were seen in both groups (pre- vs. poststudy: basal  $P <$

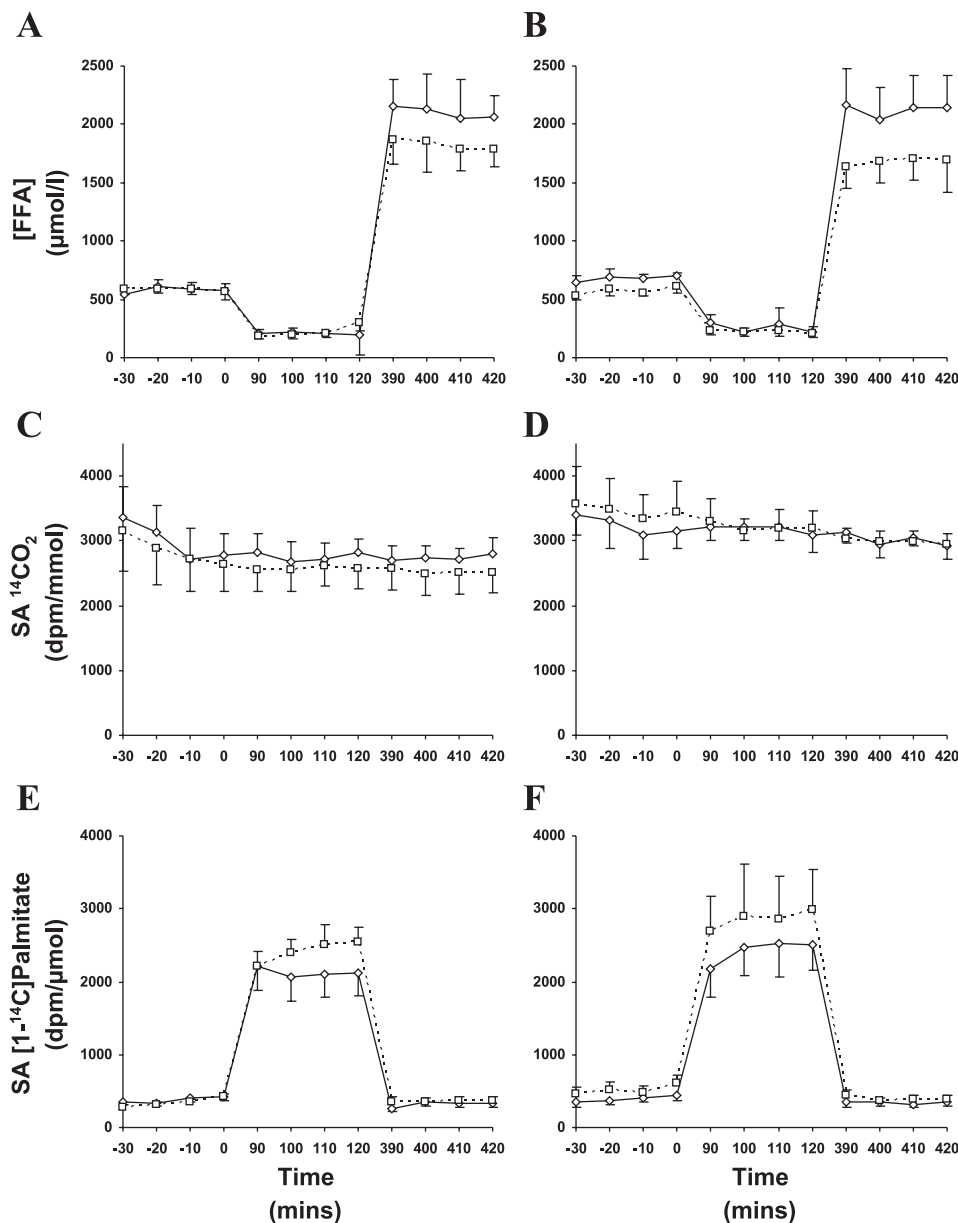


Fig. 2. Steady-state profiles during hyperinsulinemic hyperlipemic clamps performed before and after a 12-wk exercise/diet intervention. *A* and *B*: plasma free fatty acid concentrations ([FFA]) during the clamp. *C* and *D*: breath  $^{14}\text{CO}_2$  specific activities (SA). *E* and *F*: plasma  $[1-^{14}\text{C}]$ palmitate SA. Time points:  $t = -30$  to  $0$  min represents the basal clamp stage,  $t = 90$ – $120$  min represents the insulin-stimulated stage, and  $t = 390$ – $420$  min represents the insulin + intralipid/heparin stage. *A*, *C*, and *E*: EX group; *B*, *D*, and *F*: EX-HYPO group. Error bars represent SE.

Table 2. Changes in lipid metabolism during the hyperinsulinemic hyperlipemic euglycemic clamp following the intervention

Lipid Metabolism	EX		EX-HYPO	
	Pre	Post	Pre	Post
[FFA], $\mu\text{mol/l}$				
Basal	583 $\pm$ 53	584 $\pm$ 40	682 $\pm$ 41	574 $\pm$ 38 <sup>a</sup>
INS	195 $\pm$ 32 <sup>c</sup>	254 $\pm$ 85 <sup>c</sup>	283 $\pm$ 63 <sup>c</sup>	221 $\pm$ 36 <sup>c</sup>
INS + FFA	2,012 $\pm$ 220 <sup>e</sup>	1,866 $\pm$ 168 <sup>e</sup>	2,123 $\pm$ 261 <sup>e</sup>	1,764 $\pm$ 194 <sup>a,e</sup>
Net FFA <sub>ox</sub> , $\mu\text{mol}\cdot\text{kg FFM}^{-1}\cdot\text{min}^{-1}$				
Basal	4.80 $\pm$ 0.91	7.18 $\pm$ 0.70 <sup>a</sup>	6.41 $\pm$ 0.71	8.43 $\pm$ 0.62 <sup>a</sup>
INS	4.51 $\pm$ 0.62	5.58 $\pm$ 0.76 <sup>e</sup>	5.26 $\pm$ 0.91	5.78 $\pm$ 0.36 <sup>d</sup>
INS + FFA	6.08 $\pm$ 0.89	7.66 $\pm$ 0.85 <sup>a,f</sup>	6.62 $\pm$ 0.70	8.19 $\pm$ 0.59 <sup>a,g</sup>
Plasma-derived FFA <sub>ox</sub> , $\mu\text{mol}\cdot\text{kg FFM}^{-1}\cdot\text{min}^{-1}$				
Basal	1.40 $\pm$ 0.31	1.52 $\pm$ 0.40	1.92 $\pm$ 0.40	1.53 $\pm$ 0.39
INS	0.27 $\pm$ 0.06 <sup>d</sup>	0.20 $\pm$ 0.03 <sup>d</sup>	0.32 $\pm$ 0.06 <sup>d</sup>	0.37 $\pm$ 0.11 <sup>d</sup>
INS + FFA	1.90 $\pm$ 0.34 <sup>e</sup>	1.31 $\pm$ 0.15 <sup>f</sup>	2.02 $\pm$ 0.28 <sup>f</sup>	1.92 $\pm$ 0.36 <sup>e</sup>
Nonplasma lipid-derived FFA <sub>ox</sub> , $\mu\text{mol}\cdot\text{kg FFM}^{-1}\cdot\text{min}^{-1}$				
Basal	3.40 $\pm$ 0.51	5.66 $\pm$ 0.38 <sup>a</sup>	5.39 $\pm$ 0.51	5.93 $\pm$ 0.45 <sup>a</sup>
INS	4.24 $\pm$ 0.33	5.38 $\pm$ 0.37	4.94 $\pm$ 0.48	5.41 $\pm$ 0.21
INS + FFA	4.17 $\pm$ 0.54	6.35 $\pm$ 0.42 <sup>b,e</sup>	4.60 $\pm$ 0.42	6.27 $\pm$ 0.41 <sup>b,f</sup>

Data represent means  $\pm$  SE. Pre, preintervention; post, postintervention; [FFA], plasma free fatty acid concentration; FFA<sub>ox</sub>, free fatty acid oxidation; plasma-derived, FFA entering cell from plasma; nonplasma, FFA derived from intracellular or plasma triglyceride sources. Older obese men and women ( $n = 16$ ) underwent 12 wk of exercise training with (EX-HYPO) or without (EX) caloric restriction. The basal, insulin-stimulated, and lipid infusion stages of the clamp are represented by basal, INS, and INS + FFA, respectively. In the event of significant interactions between time and group, post hoc comparisons of means were performed. <sup>a</sup> $P < 0.05$  and <sup>b</sup> $P < 0.01$ , significant differences between pre and post means; <sup>c</sup> $P < 0.05$  and <sup>d</sup> $P < 0.01$ , differences between INS and basal means; <sup>e</sup> $P < 0.05$ , <sup>f</sup> $P < 0.01$ , and <sup>g</sup> $P < 0.001$ , comparisons made between INS + FFA and INS means.

0.05, INS + FFA  $P < 0.01$ ). In addition, following the interventions, nonplasma lipid-derived FFA<sub>ox</sub> during hyperlipemia was elevated above that measured during hyperinsulinemic conditions (INS + FFA vs. INS: EX  $P < 0.05$ , EX-HYPO  $P < 0.01$ ). Figure 4 highlights the changes in FFA<sub>ox</sub> during FFA-induced insulin-resistant conditions in the two dietary arms of the study; increases in net FFA<sub>ox</sub> appeared to be driven by elevations in nonplasma lipid-derived FFA<sub>ox</sub> ( $P < 0.01$ ). The same trend was true for net FFA<sub>ox</sub> during basal conditions ( $P < 0.05$ ; Fig. 5).

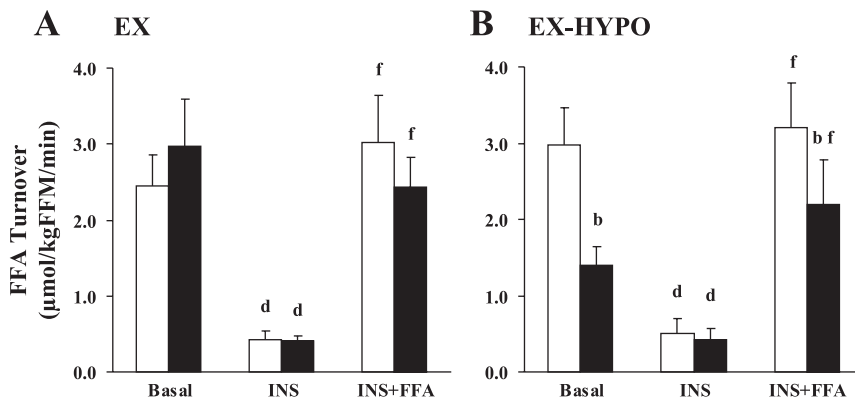
## DISCUSSION

This investigation demonstrates, for the first time, that reductions in circulating FFA concentrations during basal and hyperlipemic conditions may be attributed to decreased FFA turnover in older, obese individuals following weight loss ( $-8\%$  body wt,  $-15\%$  fat mass) induced by a long-term diet and exercise intervention. However, we have also demonstrated that improvements in peripheral tissue insulin sensitivity and FFA-induced insulin resistance are of equal magnitude following exercise training whether either a eucaloric or a

hypocaloric diet is consumed. Therefore, these improvements are independent of the amount of weight loss. Additionally, improvements in whole body FFA oxidation that were found to be driven entirely by changes in nonplasma lipid-derived FFA metabolism were also identical in each study group. Thus, it would appear that a gain of physiological function with respect to peripheral insulin resistance is driven by elevations in FFA utilization rather than decreases in plasma FFA availability.

This study was designed so that a hypocaloric diet would complement a period of increased physical activity so as to induce weight loss. The hypocaloric group demonstrated reductions in body weight that were twice as great as the exercise-only group (6.7 vs. 3.2 kg), and reductions in body fat that were nearly three times larger (5.1 vs. 1.8 kg). The exercise-only group exhibited statistically significant decreases in body weight and whole body fat mass, yet these were modest in contrast to the EX-HYPO subjects. Insulin-stimulated glucose disposal was increased equally in both study groups, indicating that the exercise training-induced improvements in insulin sensitivity are not determined by the magnitude of change in body composition. Furthermore, both groups

Fig. 3. Changes in FFA turnover across the 3 stages of the hyperinsulinemic hyperlipemic euglycemic clamp following a 12-wk exercise/diet intervention. A and B represent the EX and EX-HYPO groups, respectively. Error bars represent SE. Open bars indicate preintervention means; filled bars represent postintervention means. Basal turnover was reduced in EX-HYPO subjects only (<sup>b</sup>pre- vs. postintervention,  $P < 0.01$ ). Also, in the EX-HYPO group alone, FFA turnover was reduced during the hyperlipemic (INS + FFA) clamp stage (<sup>b</sup>pre- vs. postintervention,  $P < 0.01$ ). <sup>d</sup>Statistical differences between INS and basal means at the 1% level; <sup>f</sup>statistical differences between INS + FFA and basal means, also at the 1% level.



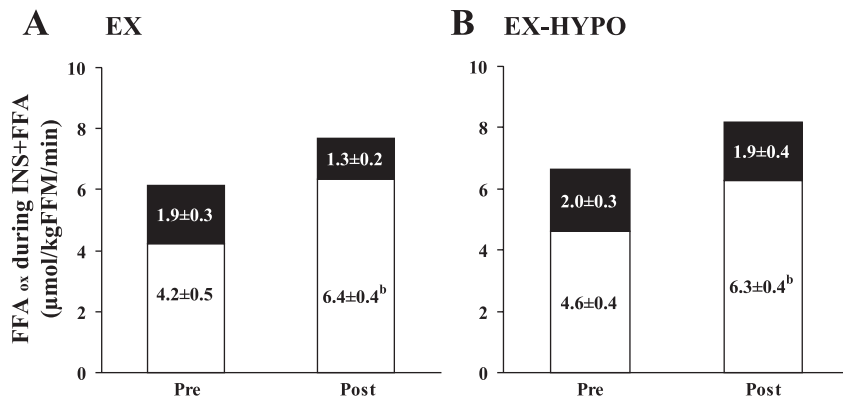


Fig. 4. Changes in FFA oxidation (FFA<sub>ox</sub>) during the hyperlipemic (INS + FFA) clamp stage following a 12-wk exercise/diet intervention. *A* and *B* represent the EX and EX-HYPO groups, respectively. Data indicate means ± SE. Total bar area represents net FFA<sub>ox</sub>. Open bars represent nonplasma lipid-derived FFA<sub>ox</sub>; filled bars represent plasma-derived FFA<sub>ox</sub>. Net FFA<sub>ox</sub> during INS + FFA was increased following the intervention in both groups (for full data, see Table 4). This was driven by increases in nonplasma FFA<sub>ox</sub> only [<sup>b</sup>pre- (Pre) vs. postintervention (Post), *P* < 0.01].

demonstrated identical attenuation in FFA-induced insulin resistance. A large body of evidence now indicates that FFAs are a key player in insulin resistance, causing lipotoxicity at elevated levels (8, 13, 17, 18, 25, 28, 36, 38, 49). The literature provides mechanistic insight for the direct and indirect effects of elevated FFA levels on insulin action. Fatty acids and their metabolites (e.g., diacylglycerol/ceramides/long-chain fatty acyl-CoAs) have been shown to activate the c-Jun NH<sub>2</sub>-terminal kinase (JNK) (1, 22) and IκB kinase-β (IKKβ) pathways (29), with each being involved in inflammation-induced insulin resistance (26, 41, 45). It has also been demonstrated that proinflammatory cytokines such as TNFα are increased during elevated FFA conditions (7) and that JNK and IKKβ activity is elevated with increased adiposity (7, 22, 50). In this study, plasma TNFα was increased during lipid infusion, yet no improvements were seen postintervention (data not shown), thus indicating a potentially minor effect of diet/exercise upon plasma cytokine involvement in FFA-induced insulin resistance. Our current study shows that basal circulating FFA concentrations and FFA turnover were reduced only in hypocaloric subjects, whereas elevations in lipid oxidation were common in both groups. In relation to the change we demonstrated in insulin sensitivity, this indicates that, indeed, elevations in FFA were inducing insulin resistance in these subjects at baseline but that such effects are not dictated simply by increased release into the circulation and therefore not by increased availability to peripheral tissue. It appears that improved insulin resistance is more likely driven by upregulation

of lipid metabolism, specifically increased nonplasma, lipid-derived, FFA oxidation. Exercise training is known to upregulate mitochondrial biogenesis and capillarization as well as increase mitochondrial oxidative capacity in skeletal muscle (12, 23, 43). Exercise also elevates intramyocellular lipid metabolism in skeletal muscle (39), and therefore, our tracer-derived measurement of nonplasma lipid-derived FFA oxidation is likely a surrogate marker of increased utilization of intramyocellular lipid (IMCL). Intracellular accumulation of lipid metabolites (e.g., diacylglycerol/ceramides/long-chain fatty acyl CoAs) is known to have a lipotoxic effect by downregulating insulin action via mechanisms outlined above, and more recently, FFA-induced insulin resistance was shown to be associated with elevations in skeletal muscle diacylglycerol, protein kinase C activity, and IKKβ activity (25). Therefore, it is likely that the exercise component of our intervention has a profound effect on insulin resistance via reduction of such moieties. Indeed, we have recently shown that multiple plasma ceramide subspecies are increased in type 2 diabetes, and these increases correlate with the level of insulin resistance (20). In addition, Dube et al. (12) and Schenk and Horowitz (39) have shown that an exercise stimulus can reduce intramuscular levels of diacylglycerol and ceramide in relation to improvements in insulin action. Further investigation is warranted to explore these effects following weight loss in humans.

It is also notable that, although whole body and intracellular lipid metabolism were elevated following both interventions, FFA oxidation during conditions of increased circulating FFA concentrations was also augmented. Remarkably, no postintervention changes were evident in plasma lipid-derived FFA oxidation. It seems that the insulin suppression and lipemia-induced elevation of plasma-derived lipid metabolism were quite normal in these obese subjects; yet it was the lipid oxidation from nonplasma (likely intracellular) sources that was found to be impaired. Therefore, the improved response of whole body lipid metabolism during times of increased lipid availability was most likely due to improvements in intracellular lipid utilization. This observation is supported by the work of Blaak et al. (4), who also reported that plasma-derived FFA oxidation was unchanged following weight loss in obese individuals. Increased utilization of IMCL following exercise has been shown previously (40) and is found in endurance-trained athletes (16); however, increased IMCL utilization during periods of elevated plasma FFA concentrations is an

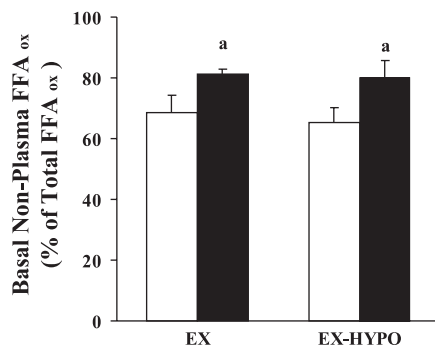


Fig. 5. Changes in basal FFA<sub>ox</sub> derived from nonplasma lipid sources following a 12-wk exercise/diet intervention. Error bars represent SE. Open bars represent preintervention means; filled bars represent postintervention means. Basal nonplasma lipid-derived FFA<sub>ox</sub> was increased following the study in both dietary groups (<sup>a</sup>pre- vs. postintervention, *P* < 0.05).

issue that requires further investigation. These findings complement evidence from our previous work showing that exercise and dietary interventions decrease IMCL content in obese insulin-resistant humans and that the improvement in whole body lipid metabolism is related to decreases in IMCL (42). A further point of interest is that, when expressed per unit of fat mass, rates of FFA turnover showed no significant changes following the interventions. Fat mass (kg) was not related to absolute basal FFA turnover ( $\mu\text{mol}/\text{min}$ ) prior to the study ( $\rho = -0.11$ ,  $P = 0.68$ ) but demonstrated an improved yet nonsignificant relationship poststudy ( $\rho = 0.43$ ,  $P = 0.09$ ). Although this trend is underpowered, it would certainly appear that, in obese insulin-resistant older adults, FFA release into the circulation does not occur in relation to the amount of whole body fat mass that is indicative of uncontrolled basal lipolysis. However, following the intervention, it appears that greater control of basal lipolysis is exhibited. Because these findings were independent of the study group, it is possible that exercise training per se influences FFA availability in relation to oxidative requirements of increased energy demands.

Our data also demonstrate significant improvements in plasma triglycerides and cholesterol, illustrating the beneficial effect of weight loss and increased physical activity on markers of cardiometabolic risk. To fully investigate in vivo lipid metabolism and understand such changes, one would require access to study hepatic lipid turnover and oxidation. Recent advances in magnetic resonance spectroscopy have reduced the methodological issues associated with studying the liver in humans. Future investigation should focus on hepatic metabolism since the liver is a key instigator of the underlying hyperglycemia common in obese, insulin-resistant patients. That said, skeletal muscle is the predominant site for glucose disposal in the adult human, and so understanding the mechanisms by which peripheral insulin resistance may arise will help clinical scientists develop therapeutic tools to combat the onset of hyperglycemia-related complications in individuals exhibiting high cardiometabolic risk.

These novel data indicate that although weight loss (via reduced caloric intake and increments in physical activity: EX-HYPO) may alleviate elevations in circulating lipids by reducing FFA turnover, this does not determine the magnitude of the gain in function with respect to peripheral tissue insulin sensitivity. In an exercising eucaloric group exhibiting modest changes in body composition, an identical gain of function with respect to insulin sensitivity was found. In conjunction with the finding that upregulation of intracellular lipid metabolism was comparable in both of our study groups, these data highlight the importance of increased physical activity for improving metabolic control. Therefore, the combination of exercise and caloric restriction is sensible because reductions in circulating FFA levels may have direct beneficial effects on lipotoxicity in other tissues not addressed by this investigation.

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