

Effects of acute lipid overload on skeletal muscle insulin resistance, metabolic flexibility, and mitochondrial performance

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Dubé JJ, Coen PM, DiStefano G, Chacon AC, Helbling NL, Desimone ME, Stefanovic-Racic M, Hames KC, Despines AA, Toledo FG, Goodpaster BH. Effects of acute lipid overload on skeletal muscle insulin resistance, metabolic flexibility, and mitochondrial performance. *Am J Physiol Endocrinol Metab* 307: E1117–E1124, 2014. First published October 28, 2014; doi:10.1152/ajpendo.00257.2014.—We hypothesized that acute lipid-induced insulin resistance would be attenuated in high-oxidative muscle of lean trained (LT) endurance athletes due to their enhanced metabolic flexibility and mitochondrial capacity. Lean sedentary (LS), obese sedentary (OS), and LT participants completed two hyperinsulinemic euglycemic clamp studies with and without (glycerol control) the coinfusion of Intralipid. Metabolic flexibility was measured by indirect calorimetry as the oxidation of fatty acids and glucose during fasted and insulin-stimulated conditions, the latter with and without lipid oversupply. Muscle biopsies were obtained for mitochondrial and insulin-signaling studies. During hyperinsulinemia without lipid, glucose infusion rate (GIR) was lowest in OS due to lower rates of nonoxidative glucose disposal (NOGD), whereas state 4 respiration was increased in all groups. Lipid infusion reduced GIR similarly in all subjects and reduced state 4 respiration. However, in LT subjects, fat oxidation was higher with lipid oversupply, and although glucose oxidation was reduced, NOGD was better preserved compared with LS and OS subjects. Mitochondrial performance was positively associated with better NOGD and insulin sensitivity in both conditions. We conclude that enhanced mitochondrial performance with exercise is related to better metabolic flexibility and insulin sensitivity in response to lipid overload.

lipids; mitochondria; skeletal muscle

CHRONIC SUBSTRATE OVERLOAD, coupled with physical inactivity, is a key mediator of the development of obesity and insulin resistance (49). It has been suggested that mitochondrial dysfunction (1, 17), perhaps a result of nutrient oversupply, particularly saturated fatty acids and/or physical inactivity, plays a key role in decreased insulin action observed in the obese state. However, there is evidence to suggest that mitochondrial content and performance are normal in insulin-resistant obese subjects (14). Although there is controversy regarding the influence of mitochondrial performance on the development of insulin resistance (17, 22), there is little debate that increased physical activity (i.e., aerobic exercise) provides a necessary stimulus for increased mitochondrial content (24), capacity for fat oxidation (6), and improved insulin sensitivity (13). Yet few studies have directly assessed the effects of lipid

oversupply on skeletal muscle insulin resistance, metabolic flexibility, and mitochondrial performance in high- vs. low-oxidative muscle (9, 40).

Using the exogenous lipid infusion model of insulin resistance (7, 26), we hypothesized that excess fatty acids would be preferentially oxidized in endurance-trained athletes and that insulin resistance would be attenuated owing to a relative preservation of nonoxidative glucose disposal. We reasoned that these results would be directly related to greater mitochondrial respiratory capacity in these subjects. We demonstrate that despite similar declines in insulin sensitivity with lipid overload, individuals with a higher mitochondrial respiratory capacity are able to increase reliance on whole body fat oxidation at the expense of carbohydrate oxidation and maintain higher nonoxidative glucose disposal, presumably glycogen storage. Nonoxidative glucose disposal (NOGD), as well as insulin sensitivity during lipid overload and glycerol control, was correlated to carbohydrate-supported mitochondrial respiration.

METHODS

Study Subjects

Young (20–35 yr) lean endurance-trained (LT; BMI <25 kg/m², *n* = 14), obese sedentary (OS; BMI = 30–35 kg/m², *n* = 14), and lean sedentary (LS; BMI <25 kg/m², *n* = 13) males and females were recruited through print advertisements in the Pittsburgh, PA, area. LT subjects reported structured exercise 3–5 days/wk for ~2 yr, with <3 yr of interrupted training. Subjects were medically screened at the University of Pittsburgh's Clinical and Translational Research Center and gave written consent to the protocol that was approved by the University of Pittsburgh Institutional Review Board.

Circulating Lipids, Glucose, and Insulin

Circulating plasma free fatty acids (FFAs) were quantified using GC-FID (27) and triacylglycerols measured by standardized hospital laboratory procedures. Plasma glucose and insulin were measured by glucose oxidase and ELISA, respectively.

Body Composition and Maximal Aerobic Capacity

Total and regional fat mass and fat-free mass (FFM) were assessed by dual-energy X-ray absorptiometry (Lunar, GE Lunar Prodigy, Encore 2005 software, version 9.30) (12). Maximal aerobic capacity ($\dot{V}O_{2\max}$) was determined by a graded exercise test on a cycle ergometer (12).

Insulin Sensitivity and Lipid Oversupply

Insulin sensitivity was determined using a 6-h hyperinsulinemic (80 mU·m⁻²·min⁻¹) euglycemic (90 mg/dl, 20% dextrose) clamp

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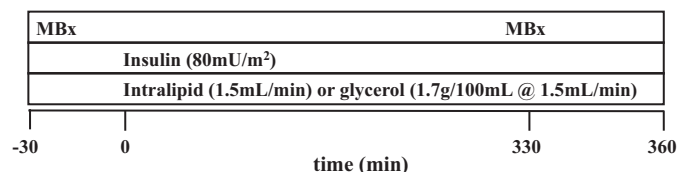


Fig. 1. Study design. MBx, muscle biopsy.

method (13) under one of the two experimental conditions (26) separated by 2 wk (Fig. 1). Subjects refrained from exercise 48 h prior to the clamp. Glucose infusion rate (GIR), which was necessary to maintain euglycemia, was calculated from the last 30 min of steady state.

Experiment 1 (lipid oversupply). Subjects were infused with exogenous lipid [1.5 ml/min, 20% fat emulsion (30% soybean oil, 1.2% egg yolk phospholipids, 1.7% glycerol, and water); Baxter Healthcare, Deerfield, IL].

Experiment 2 (control). Saline-glycerol (1.7 g/100 ml, 1.5 ml/min) infusion was used as a control. Glycerol was added to account for the glycerol content in the lipid infusion and to ascribe the metabolic defects to FA oversupply (*experiment 1*) alone (44).

Whole Body Substrate Utilization

Indirect calorimetry (Parvo Medics, Sandy, UT) was employed with urinary nitrogen corrections to calculate rates of postabsorptive and insulin-stimulated fat and glucose oxidation and NOGD.

Skeletal Muscle Biopsy and Tissue Analysis

Biopsy. Prior to the clamp procedure, a percutaneous muscle biopsy of the vastus lateralis was performed (12). A second muscle biopsy was performed at +340 min of the clamp, ~2 cm proximal from the first biopsy site.

Tissue analysis. EX VIVO MITOCHONDRIAL RESPIRATION. For permeabilized muscle fiber bundles, ~5 mg of biopsy sample was permeabilized with saponin and prepped for high-resolution O_2 flux, as described previously (10). For the mitochondrial respiration protocol, measurement of oxygen consumption was conducted at 37°C in the oxygen concentration range of 220 to 150 nmol O_2 /ml, as described previously in detail (10).

PROTEIN ANALYSIS. Western blots were used to determine relative protein content in a semiquantitative manner [oxidative phosphorylation (OXPHOS), Mitosciences no. ab110411, p-Akt (Ser⁴⁷³); Cell Signaling Technology no. 9271 and p-GSK-3 β (Ser⁹); Cell Signaling Technology no. 9336] (13), quantified by densitometry (ImageJ; National Institutes of Health), and normalized to total protein (pan-Akt; Cell Signaling Technology no. 2920; GSK-3 β , Cell Signaling Technology no. 9315) or β -actin (Cell Signaling Technology no. 4967). Internal loading control was added to adjust for gel-to-gel variability.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS for Mac, version 20). When no differences were observed for preinfusion values (control and lipid oversupply), values were averaged to create a “baseline” variable. Baseline group differences were assessed using a one-way analysis of variance (ANOVA). A two-way repeated-measures ANOVA was used to determine main (group, treatment) and interaction effects. When interaction effects were observed, between-groups effects were determined by one-way ANOVA on percent change (Δ pre- vs. postinfusion) and within-group infusion effects determined by paired-samples *t*-test. Pearson’s correlation analysis tested the relationship between mitochondrial capacity, substrate utilization, and insulin sensi-

tivity. Statistical significance was assumed at $P < 0.05$. Data are presented as means \pm SE.

RESULTS

Study Subjects

Baseline subject characteristics are presented in Table 1. By design, OS had higher ($P < 0.01$) BMI, body weight, and fat mass compared with LS and LT, whereas FFM was similar. The LT subjects had greater cardiorespiratory fitness ($\dot{V}O_{2max}$) ($P < 0.01$).

Blood Chemistry

Baseline plasma values obtained during the screening visit are presented in Table 1. Both glucose and insulin were higher ($P < 0.05$) in OS, whereas total nonesterified FFAs and triglycerides were similar between the groups. Blood glucose was similar in the control and lipid conditions; however, insulin levels were lower with lipid compared with control (Table 2). Hyperinsulinemia decreased circulating FFAs ($93.2 \pm 1.8\%$, $P < 0.01$) compared with baseline. With lipid oversupply at steady state, FFAs were elevated compared with the control condition ($P < 0.01$; Table 2) but not different from baseline (i.e., preinfusion).

Insulin Sensitivity and Metabolic Flexibility in Response to Lipid Overload

In the control condition, obese subjects demonstrated lower insulin sensitivity (LT, 16.1 ± 2.8 ; LS, 14.7 ± 2.8 ; OS, 11.8 ± 3.7), as determined by the GIR normalized to FFM ($mg \cdot kg^{-1} \cdot min^{-1}$), and rates of NOGD (Fig. 2 and Table 3). Absolute rates of carbohydrate and fat oxidation were similar between the groups (Table 3), and yet the relative proportion of glucose oxidation was higher in OS (Fig. 2).

In response to lipid infusion, peripheral insulin sensitivity was decreased similarly in all subjects ($-41.8 \pm 2.1\%$, $P < 0.01$; Fig. 2A). Absolute rates of carbohydrate oxidation were decreased in response to lipid oversupply in all subjects ($-34.5 \pm 5.5\%$; Table 3). However, change in carbohydrate oxidation as a proportion of total glucose disposal was decreased only in LT ($-22.6 \pm 17.5\%$) but not LS ($36.0 \pm 10.4\%$) or OS ($21.2 \pm 12.3\%$) (Fig. 2B). Rates of whole body fat oxidation increased to a greater degree in LT ($13.3 \pm 1.3\%$) compared with LS ($5.0 \pm 1.2\%$) and tended to be higher than the increase in OS ($7.4 \pm$

Table 1. Baseline subject characteristics

	LT	LS	OS
<i>n</i> (Males/females)	10 (5/5)	10 (5/5)	10 (4/6)
Age, yr	27.5 \pm 1.4 ^{a,b}	24.3 \pm 1.1 ^b	29.5 \pm 1.4 ^a
BMI, kg/m ²	22.0 \pm 0.6 ^b	23.0 \pm 0.1 ^b	36.4 \pm 1.2 ^a
FFM, kg	53.3 \pm 2.8	50.1 \pm 3.0	59.9 \pm 4.1
FM, kg	12.0 \pm 1.1 ^b	18.0 \pm 1.4 ^b	45.3 \pm 3.6 ^a
Glucose, mg/dl	86.7 \pm 1.7 ^b	84.3 \pm 1.3 ^b	92.4 \pm 2.3 ^a
Insulin, μ U/ml	2.2 \pm 0.4 ^b	3.9 \pm 0.7 ^b	7.6 \pm 1.4 ^a
Insulin, μ M	486.9 \pm 84.6	408.0 \pm 55.4	393.7 \pm 37.0
Triglyceride, mg/dl	49.4 \pm 5.2	54.4 \pm 12.2	78.0 \pm 13.7
$\dot{V}O_{2max}$, ml \cdot kg FFM ⁻¹ \cdot min ⁻¹	70.6 \pm 2.5 ^a	52.6 \pm 1.8 ^b	44.0 \pm 2.3 ^c

Data are means \pm SE. BMI, body mass index; FFM, fat-free mass; FM, fat mass; FFA, free fatty acids; LT, lean trained; LS, lean sedentary; OS, obese sedentary; $\dot{V}O_{2max}$, maximal aerobic capacity. ^{a,b,c} $P < 0.05$, baseline difference (1-way analysis of variance); noncorresponding letters are different.

Table 2. Blood chemistry in control and lipid oversupply at steady state

	LT		LS		OS	
	Control	Lipid	Control	Lipid	Control	Lipid
Glucose, mg/dl	87.2 ± 2.0	89.2 ± 1.7	85.4 ± 1.0	89.4 ± 1.3	89.3 ± 1.3	90.5 ± 1.4
Insulin, uU/ml	75.6 ± 8.9	70.4 ± 6.1*	96.6 ± 18.2	60.8 ± 7.2*	138.4 ± 14.4	84.3 ± 19.4*
FFA, μM	19.5 ± 1.5	411.2 ± 26.3*	23.6 ± 0.2	498.8 ± 48.5*	31.1 ± 0.4	324.0 ± 36.3*

Data are means ± SE. * $P < 0.01$, control vs. lipid effect.

1.1%, $P = 0.07$; Table 3). Post hoc analysis demonstrated that the absolute rates of fat oxidation were higher in LT and OS compared with LS (Table 3). Absolute rates of NOGD were decreased in all subjects; this decrease, however, was significantly attenuated in LT ($-26.6 \pm 6.4\%$) compared with OS and LS (-51.9 ± 5.6 and $-50.0 \pm 4.2\%$, respectively) (Table 3). Moreover, the percent of NOGD to total glucose disposal in response to lipid was increased in LT (12.6 ± 8.1) yet de-

creased in both LS ($-17.7 \pm 6.1\%$) and OS ($-14.2 \pm 8.9\%$) (Fig. 2C).

Mitochondrial Performance and Lipid Overload

Overall, non-insulin-stimulated O_2 flux tended to be highest in LT for all respiratory states (Fig. 3). During fasted conditions, the respiratory control ratio (RCR; state 3/state 4 respiration), an index of uncoupling, was similar among the groups. Correlational analyses for baseline respiratory measurements and body composition, maximal aerobic fitness, insulin sensitivity, and substrate kinetics are reported in Table 4.

Hyperinsulinemia increased state 4 respiration in all groups; however, the relative increase was not different between the groups (LT, 26.4 ± 17.3 ; LS, 15.4 ± 15.5 ; OS, $35.2 \pm 12.5\%$). State 3 and maximal uncoupled respiration were decreased equally across groups (Fig. 3, A–C). RCR was decreased ($P < 0.05$) in all subjects, mediated by the increase in state 4 respiration. With lipid oversupply, state 4 respiration did not increase as observed in the control condition. However, state 3 and uncoupled respiration were decreased in each group to a degree similar to that observed in the control condition. RCR was decreased with lipid oversupply and not different from the insulin-stimulated control condition. OXPHOS protein content was not different between the groups.

Proteins Involved in Insulin Sensitivity and Metabolic Flexibility

Baseline Akt and GSK-3 β levels were similar between the groups (Fig. 4, A–D) and between conditions. Insulin-stimulated Akt phosphorylation (p-Akt) was lower ($P = 0.02$) in OS compared with LT in the control condition (Fig. 4, A and C) and was correlated with GIR ($r = 0.40$, $P = 0.02$). p-Akt was increased to a similar degree in each condition and was correlated to NOGD ($r = 0.46$, $P = 0.01$) in the presence of lipid oversupply. The responses for GSK-3 β phosphorylation as a marker of muscle glycogen synthesis were similar to that of p-Akt (Fig. 4, B and D).

DISCUSSION

The overall purpose of this study was to determine the potential for high-oxidative muscle to prevent or attenuate the effects of acute lipid oversupply on insulin sensitivity and metabolic flexibility. We further sought to determine whether the effects of lipid oversupply were similar in low-oxidative (sedentary) muscle set on a background of obesity, particularly with respect to substrate utilization and mitochondrial performance. We demonstrated that although endurance-trained subjects are not immune to lipid-induced insulin resistance, they maintained significantly higher whole body fat oxidation as

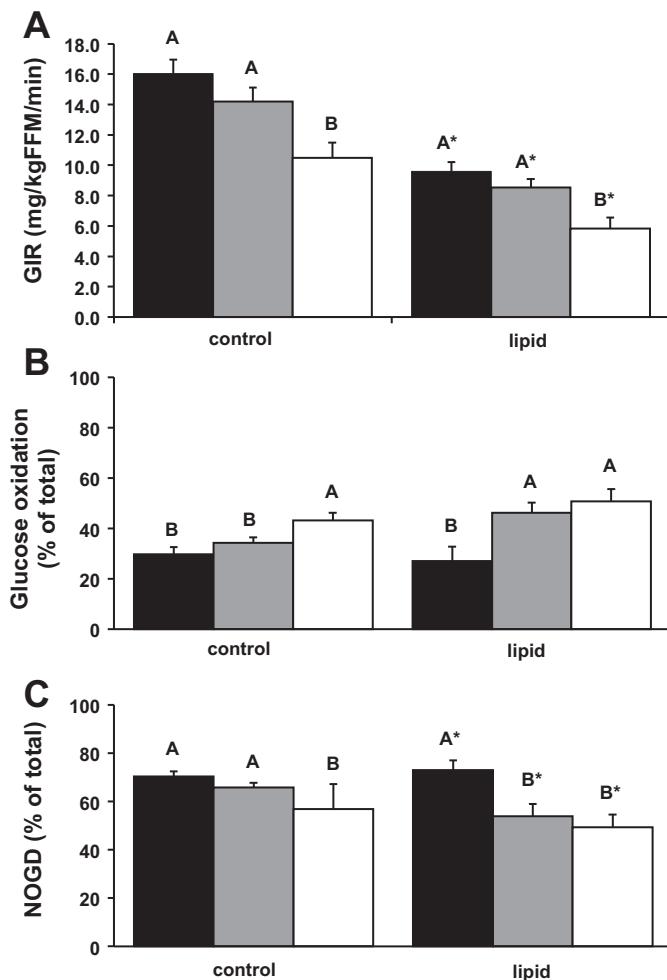


Fig. 2. Insulin sensitivity and glucose metabolism. A: total glucose disposal as determined by the glucose infusion rate (GIR), normalized to fat-free mass (FFM), during steady-state was determined by the hyperinsulinemic euglycemic clamp described in METHODS. B and C: glucose oxidation (B) and nonoxidative glucose disposal (NOGD; C), calculated as %total glucose disposal. Black bars, lean trained; gray bars, lean sedentary; open bars, obese sedentary. ^{A,B,C} $P < 0.05$, group difference; noncorresponding letters are different. * $P < 0.05$ different from control. Data are means ± SE.

Table 3. *Substrate utilization*

	LT		LS		OS	
	Control	Lipid	Control	Lipid	Control	Lipid
Glucose oxidation, ml·kg FFM ⁻¹ ·min ⁻¹	5.29 ± 0.39	2.17 ± 0.41*	5.33 ± 0.43	3.91 ± 0.40*	4.69 ± 0.41	2.96 ± 0.49*
NOGD, ml·kg FFM ⁻¹ ·min ⁻¹	10.35 ± 0.57 ^a	7.69 ± 0.84*	9.41 ± 0.64 ^a	4.63 ± 0.45*	5.81 ± 0.78 ^b	2.87 ± 0.53*
Fat oxidation, ml·kg FFM ⁻¹ ·min ⁻¹	0.35 ± 0.16	1.35 ± 0.17*	0.22 ± 0.11	0.54 ± 0.11*	0.37 ± 0.11	1.02 ± 0.11*

Data are means ± SE. NOGD, nonoxidative glucose disposal. * $P < 0.01$, control vs. lipid effect. ^{a,b} $P < 0.05$, group effect; noncorresponding letters are different.

well as a relative preservation of NOGD. Hyperinsulinemia modestly increased state 4 respiration independent of phenotype yet decreased both coupled and uncoupled respiration. In response to lipid overload, only state 4 was affected. Lipid-induced insulin resistance could not be attributed to alterations in either Akt or GSK3 phosphorylation.

The key experimental paradigm in our study was to employ a model of acute lipid oversupply to induce insulin resistance

(9, 26, 40). Whether or not high-oxidative muscle is “protected” from the effects of lipid oversupply is controversial. While Phielix et al. (40) demonstrated an attenuation of lipid-induced insulin resistance in chronic endurance-trained athletes compared with lean sedentary controls, Chow et al. (9) demonstrated similar decreases in insulin sensitivity that were independent of training status. The latter study is in line with our data suggesting that lipid oversupply results in comparable

Fig. 3. Mitochondrial respiration. Skeletal muscle fiber bundles were prepared from biopsy samples, as described in METHODS. Mean O₂ flux, normalized to dry tissue weight (mg), for preinfusion (black bars) and postinfusion (open bars) is presented for the control (A–C) and lipid oversupply (D–F) conditions. ^{A,B} $P < 0.05$, group difference; noncorresponding letters are different. * $P < 0.05$ different from preinfusion. Data are means ± SE. LT, lean trained; LS, lean sedentary; OS, obese sedentary.

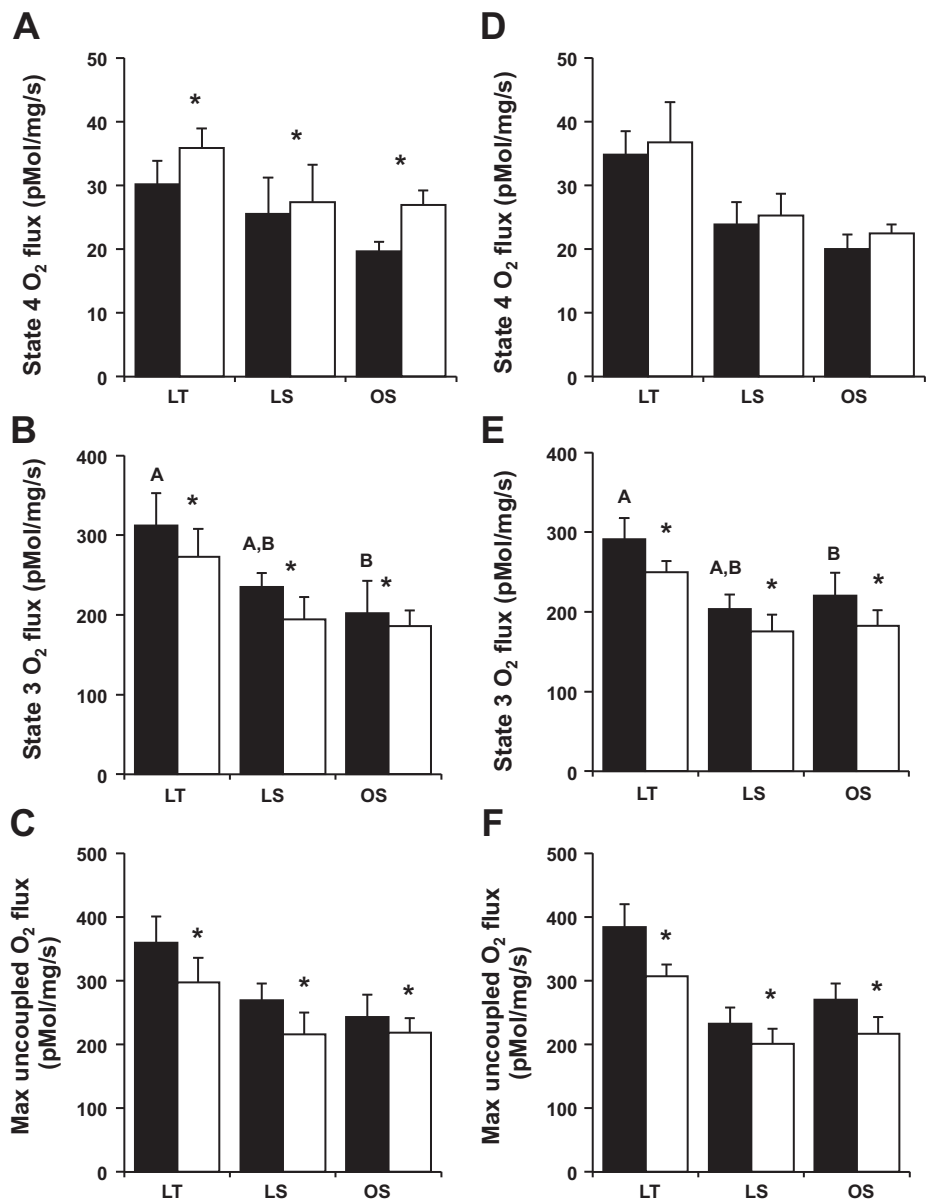


Table 4. Correlational analyses for baseline respiratory measurements and body composition, maximal aerobic fitness, insulin sensitivity, and substrate kinetics

	State 4	State 3 Max	Uncoupled
BMI, kg/m ²	$r = -0.42^*$	$r = -0.57^\dagger$	$r = -0.48^*$
FM, kg	$r = -0.47^*$	$r = -0.59^\dagger$	$r = -0.48^*$
FFM, kg	$r = 0.06$	$r = -0.04$	$r = 0.05$
$\dot{V}O_{2\max}$, ml·kg			
FFM ⁻¹ ·min ⁻¹	$r = 0.49^*$	$r = 0.70^\dagger$	$r = 0.71^\dagger$
Control			
Fat oxidation, mg·kg			
FFM ⁻¹ ·min ⁻¹	$r = -0.01$	$r = -0.23$	$r = -0.15$
Carbohydrate oxidation,			
mg·kg FFM ⁻¹ ·min ⁻¹	$r = 0.12$	$r = 0.35$	$r = 0.27$
NOGD, mg·kg			
FFM ⁻¹ ·min ⁻¹	$r = 0.38$	$r = 0.56^\dagger$	$r = 0.44^*$
GIR, mg·kg			
FFM ⁻¹ ·min ⁻¹	$r = 0.28$	$r = 0.49^\dagger$	$r = 0.36$
Lipid			
Fat oxidation, mg·kg			
FFM ⁻¹ ·min ⁻¹	$r = 0.05$	$r = 0.05$	$r = 0.19$
Carbohydrate oxidation,			
mg·kg FFM ⁻¹ ·min ⁻¹	$r = 0.02$	$r = -0.08$	$r = -0.07$
NOGD, mg·kg			
FFM ⁻¹ ·min ⁻¹	$r = 0.42^*$	$r = 0.63^\dagger$	$r = 0.70^\dagger$
GIR, mg·kg			
FFM ⁻¹ ·min ⁻¹	$r = 0.30$	$r = 0.46^*$	$r = 0.48^*$

* $P \leq 0.05$; $^\dagger P \leq 0.01$.

decreases in glucose disposal in lean trained and untrained subjects. We extend previous findings and report similar decreases in insulin sensitivity in obese sedentary subjects with lipid oversupply. One potential explanation for this apparent discrepancy between the studies may be related to methodological differences. The study by Phielix et al. (40) included a coinfusion of heparin (26) in conjunction with the lipid emulsion, thereby raising circulating FFA levels to supraphysiological levels (>1 mM). In contrast, we did not coinfuse heparin, and thus our data mimic high physiological FFA levels relative to insulin-suppressed levels during hyperinsulinemic control conditions (9). Circulating FFA levels in the current study are in line with postprandial FFA levels following a high-fat meal of similar fatty acid composition (29, 41). Together, these data suggest that methodological differences may play a key role in the interpretation of studies involving lipid-induced insulin resistance.

Had we measured only rates of total insulin-stimulated glucose disposal, we would have concluded that high mitochondrial oxidative capacity fails to protect against lipid-induced insulin resistance. However, we demonstrate a greater shift toward fat oxidation at the expense of carbohydrate oxidation, with a relative maintenance of NOGD in endurance-trained athletes compared with obese subjects with lower oxidative muscle (30, 46), which is consistent with the notion that improved insulin sensitivity with weight loss or exercise is due largely to enhanced glucose storage with lesser effects on glucose oxidation (18). We demonstrate that a greater degree of insulin-stimulated Akt activation and total glucose transport were associated with higher rates of NOGD in the presence of lipid oversupply (40). Higher rates of fat oxidation with obesity in the presence of lipid oversupply support human exercise studies whereby FFAs are increased and rates of fatty acid oxidation are higher in obese vs. lean subjects (19). Our study

provides additional novel data that although obese subjects had a similar reduction in carbohydrate oxidation in response to lipid oversupply compared with the athletes in our study, they were less capable of increasing fat oxidation and preserving glucose storage in the presence excess lipid. Thus, our study provides additional experimental evidence supporting the metabolic inflexibility in obesity.

The causal role for mitochondrial function in insulin sensitivity is controversial (17, 22). However, there is little debate that chronic exercise improves both insulin action (6, 13) and mitochondrial capacity (5, 12, 39). In agreement with Fisher-Wellman et al. (14), we did not observe any alterations in mitochondrial content in either muscle from sedentary lean and obese participants, as demonstrated by OXPHOS protein content and similar FCCP-induced maximal uncoupling. Our data suggest that carbohydrate-supported maximal coupled respiration is increased with exercise training and is correlated with insulin sensitivity, further demonstrating the effects of exercise on mitochondrial performance and insulin sensitivity. A novel finding of this study was that insulin alone increased state 4 respiration independently of phenotype yet decreased both maximal coupled and uncoupled respiration, resulting in a decrease in the respiratory control ratio. This novel finding suggests that hyperinsulinemia uncouples the inner mitochondrial membrane, perhaps to promote better matching of substrate supply (elevated pyruvate) and energy production [increased reducing equivalents (NADH)] (28, 47) and/or glycolytic flux (31). Moreover, hyperinsulinemia may directly mediate impairments in the electron transport system, as evidenced by the decrease in maximal uncoupled respiration, through the increased substrate flux (31), accelerated electron leak, and production of reactive oxygen species (42).

Acute lipid oversupply directly and specifically inhibited state 4 respiration, suggesting an inhibition of glucose oxidation due to the presence of excess fatty acids (25). Yet, lipid oversupply does not appear to further impair carbohydrate-supported maximal coupled or uncoupled respiration within this cohort of subjects across a range of oxidative capacity. This is supported by the work of Daniele et al. (11), who demonstrated that reducing circulating FFA levels increases carbohydrate supported ATP production in both type 2 diabetic patients as well as normal-glucose-tolerant subjects. We suggest that mitochondrial performance is not impaired to a greater degree in low-oxidative muscle confounded with obesity (14, 23) within the context of excess lipid exposure. This distinction of carbohydrate- vs. mixed-substrate on mitochondrial performance is important, as previous studies have suggested that mitochondrial respiration is not enhanced by exercise training (40) or impaired by acute lipid oversupply (3) using mixed (lipid and carbohydrate) substrates. Our demonstration of negative associations between state 4 respiration and whole body carbohydrate kinetics, as well as lipid-induced insulin resistance specifically in state 4, suggest a state of physiological insulin resistance (25). In other words, in the face of excess lipid, mitochondria preferentially switch from carbohydrate to fatty acid as the preferred substrate, with no defects observed in insulin signaling (25). This switch is based on classical Randle cycle interactions and has been described as metabolic feedback by Hoy et al. (25). This “physiological insulin resistance” is a normal response and occurs in other physiological contexts, for example, during prolonged fasting.

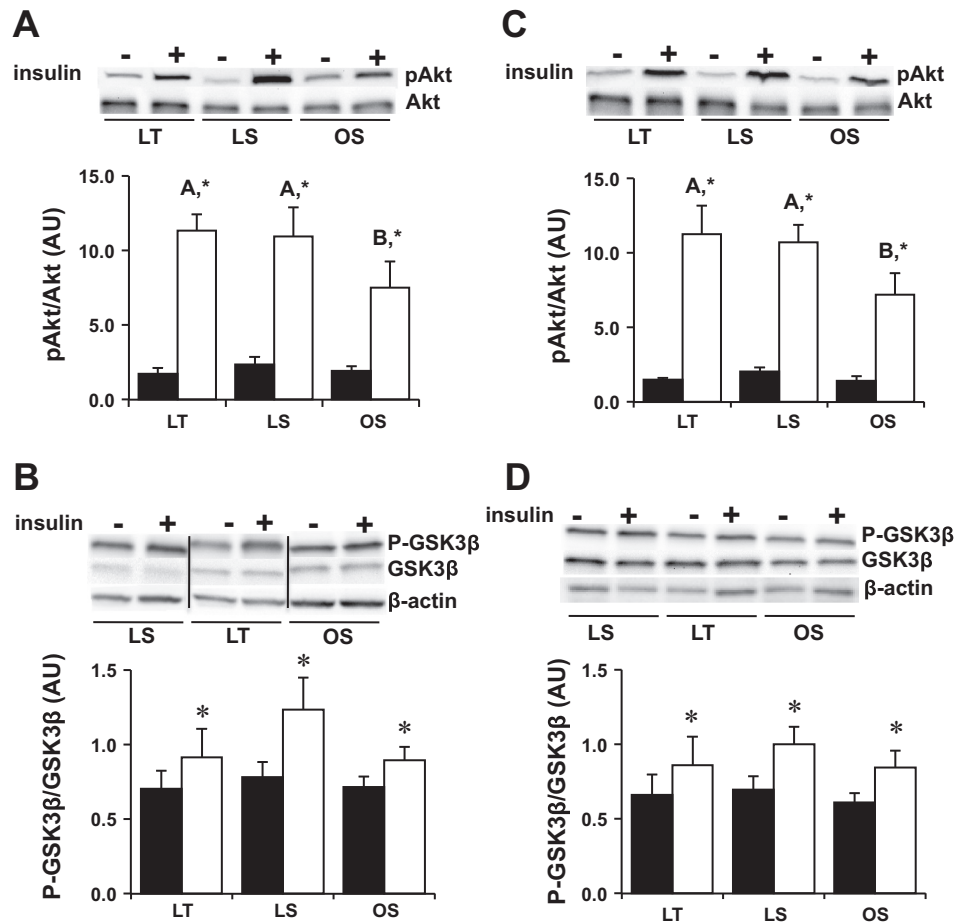


Fig. 4. Skeletal muscle proteins related to insulin sensitivity and metabolic flexibility. Akt phosphorylation (p-Akt; A and C), and p-GSK-3 β (B and D) measured by Western blot, as described in METHODS. Data represent preinfusion (black bars) and insulin-stimulated (open bars) quantified values for control (A and C) and lipid (C and D) infusions, respectively. Representative immunoblots arranged to coincide with figures. ^{A,B} $P < 0.05$, group difference; noncorresponding letters are different. $*P < 0.05$ different from preinfusion. Data are means \pm SE. Black lines through immunoblots represent spliced images to follow group and treatment order.

We speculate that this differs mechanistically from pathological insulin resistance (34), which is characterized by intramyocellular lipid accumulation (38) and impaired insulin signaling (45, 48). Thus, our data are more in line with the substrate competition hypothesis (Randle cycle) (43), whereby fat oxidation is maintained (3) to a relatively greater degree or, as in the case of this model, enhanced at the expense of carbohydrate oxidation. These data are further strengthened due to the pre- and postinfusion biopsy study design not employed by other studies (3, 40).

Alterations in proximal insulin signaling concomitant with insulin resistance have been observed in some (2) but not all studies of insulin-resistant obese subjects (4, 32, 33). The current study tends to support the notion that insulin-stimulated Akt phosphorylation is inhibited in low-oxidative muscle and regulated particularly by the degree of obesity (2). However, the model system of acute lipid oversupply within the physiological range (9) does not result in lipid metabolite (i.e., diacylglycerol, ceramide) accumulation (8, 26, 37, 50), which is known to mediate insulin signaling. Thus, we provide compelling evidence in humans of physiological insulin resistance or metabolic feedback with lipid oversupply. In other words, we demonstrate insulin resistance without defects in insulin signaling. The positive correlations between Akt-phosphorylation and carbohydrate kinetics, as well as insulin sensitivity, provide evidence that Akt phosphorylation may be used as a surrogate marker for insulin sensitivity under certain

conditions. This hypothesis is supported by our demonstration that the correlations between Akt phosphorylation, carbohydrate kinetics, and insulin sensitivity are lost under the lipid infusion condition. These data are in accord with studies in both rodents (25) and cell culture (21) demonstrating a disconnect between insulin-stimulated Akt phosphorylation and acute lipid oversupply. Together, these data highlight the complexity of proximal insulin signaling and pathophysiology of insulin resistance (48). We next investigated whether glycogen synthase kinase-3 β (GSK-3 β), which is downstream of Akt and is a regulator of glycogen synthase, may influence our observations of enhanced NOGD in high-oxidative muscle in the face of acute lipid oversupply. GSK-3 β phosphorylation was not different in high- or low-oxidative muscle in this study in response to hyperinsulinemia alone (control) (16). Interestingly, however, non-insulin-stimulated GSK-3 β phosphorylation was negatively associated with state 4 carbohydrate-supported respiration. These data further emphasize the importance of mitochondrial performance and glycogen metabolism within the context of insulin sensitivity (20). In contrast to previous reports, we did not observe any inhibition by lipid on the capacity for insulin-stimulated GSK-3 β phosphorylation in low-oxidative muscle (40). The discrepancy in results is likely methodological in nature. One hypothesis is that higher levels of insulin may be sufficient to overcome the deleterious effects of acute lipid oversupply (36). Alternatively, it could be hypothesized that acute elevations of circulating fatty acids

within the physiological range are insufficient to alter insulin-stimulated GSK-3 β phosphorylation (15). Together, these data provide evidence for normal insulin signaling within the context of physiological insulin resistance and suggest a negative feedback mechanism (25) directed potentially toward intracellular glucose transport machinery and function (35).

In summary, using a model of physiological insulin resistance, we demonstrate similar decreases in total insulin-stimulated glucose disposal in exercise-trained and sedentary subjects with and without confounding obesity. These defects do not appear to be mediated directly through alterations in insulin signaling. Endurance training dramatically alters substrate kinetics in response to lipid overload. With exercise, excess lipid is preferentially oxidized at the expense of glucose oxidation and, by extension, total glucose disposal. Moreover, NOGD is relatively preserved in endurance-trained athletes but not in obese sedentary subjects, further emphasizing the importance of chronic physical activity in the handling of substrate overload and maintaining better metabolic flexibility. Our data support the notion of impaired mitochondrial performance as a key mediator of the insulin resistance. We propose that enhanced mitochondrial performance associated with chronic physical activity is directly associated with improved glycogen metabolism, which is a prominent feature of insulin sensitivity.

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DISCLOSURES

The authors have no conflicts of interest, financial or otherwise, to declare.

AUTHOR CONTRIBUTIONS

J.J.D., F.G.T., and B.H.G. conception and design of research; J.J.D., P.M.C., G.D., A.C.C., N.L.H., M.D., M.S.-R., K.C.H., A.A.D., and F.G.T. performed experiments; J.J.D. analyzed data; J.J.D., P.M.C., and B.H.G. interpreted results of experiments; J.J.D. prepared figures; J.J.D. drafted manuscript; J.J.D., P.M.C., and B.H.G. edited and revised manuscript; J.J.D. and B.H.G. approved final version of manuscript.

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