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# Short-term leptin treatment increases fatty acids uptake and oxidation in muscle of high fat–fed rats☆

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**Abstract**

The purpose of this study was to measure the effects of short-term (10 days) leptin treatment on insulin sensitivity as it pertains to fatty acid (FA) uptake, oxidation, and muscle triglyceride (mTG) synthesis in animals that have been administered a high-fat (HF) diet for 3 months. Male Wistar rats were randomly assigned to 1 of 4 groups. One group was fed a control diet (CON) and 3 groups were fed a HF diet. The HF and HF-leptin (HF-LEP) groups were fed the HF diet ad libitum and the amount of food eaten by the HF–pair fed (HF-P) group was equal to that of the HF-LEP group. At the end of the dietary period, rats were injected daily either with saline (CON, HF, HF-P) or with leptin (HF-LEP; 10 mg · $kg^{-1}$ · $d^{-1}$) for 10 days before hindlimb perfusion. The perfusate contained 600 $\mu$mol/L palmitate traced with [$^{14}$C]palmitate, 9 mmol/L glucose, and 100 $\mu$U/mL insulin. As dictated by the protocol, energy expenditure was not significantly different ($P > .05$) between HF-LEP and HF-P. Palmitate uptake and oxidation as well as mTG synthesis were greater ($P < .05$) in HF (9.8 ± 0.3, 2.0 ± 0.1, and 1.9 ± 0.2 nmol · $min^{-1}$ · $g^{-1}$) than in CON (8.0 ± 0.4, 1.4 ± 0.1, and 1.1 ± 0.1 nmol · $min^{-1}$ · $g^{-1}$) and this was associated with higher levels of mTG in HF. Palmitate uptake and oxidation were higher ($P < .05$) in HF-LEP (10.3 ± 0.6 and 2.0 ± 0.1 nmol · $min^{-1}$ · $g^{-1}$) than in HF-P (8.3 ± 0.5 and 1.5 ± 0.2 nmol · $min^{-1}$ · $g^{-1}$, $P < .05$), but mTG synthesis and mTG levels were not changed significantly by leptin treatment ($P > .05$). High-fat feeding decreased glucose uptake by 41% when compared with CON (2.4 ± 0.4 vs 4.1 ± 0.4 $\mu$mol · $h^{-1}$ · $g^{-1}$; $P < .05$) but pair feeding alone (4.7 ± 0.4 $\mu$mol · $h^{-1}$ · $g^{-1}$) or leptin treatment (3.8 ± 0.3 $\mu$mol · $h^{-1}$ · $g^{-1}$) similarly prevented the HF diet–induced decrease in glucose uptake. These data indicate that short-term leptin treatment in HF-fed rats alters muscle FA metabolism by increasing FA uptake and oxidation relative to pair feeding alone. This results in a decrease in the FA esterification-oxidation ratio.


## 1. Introduction

The impairment of insulin-stimulated glucose uptake by high concentrations of circulating fatty acids (FA) induced by either intralipid infusion or adherence to a high-fat (HF) diet has been well documented [1-4] and suggests that alterations in muscle FA metabolism may be involved in the development of insulin resistance. In line with this notion, muscle triglyceride (mTG) accumulation has been shown to negatively correlate with whole-body insulin-stimulated glucose uptake [5,6]. Although mTG accumulation can occur because of alterations in FA uptake, FA oxidation and/or FA esterification into mTG under both basal and insulin-stimulated conditions, it is not clear which of these metabolic pathways are the most affected by a HF diet. We have demonstrated that 3 weeks of HF feeding (65% fat-derived energy) is accompanied by mTG accumulation, a decrease in glucose uptake, and an increase in FA oxidation in muscle perfused under insulin-stimulated conditions [7]. These results suggest that resistance to the actions of insulin is present in muscle of HF-fed rats not only as it pertains to glucose uptake but also to the antioxidative

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actions of insulin on FA disposal [7]. However, it is not known whether the increase in mTG associated with HF feeding of a longer duration would be associated with further alterations in muscle FA kinetics under insulin-stimulated conditions.

Although it is generally accepted that alterations in FA metabolism are involved in the development of muscle insulin resistance, clinical strategies aimed at preventing the development of these metabolic pathologies are still highly debated [8]. Treatment with leptin is one of the pharmacologic interventions that have been studied because of its ability to improve insulin sensitivity in muscle [9]. Leptin is the 16-kd product of the *ob* gene, which is secreted by adipocytes in proportion to the amount of stored lipid [10]. Early studies have shown that leptin decreases food intake and regulates energy expenditure possibly via inhibition of neuropeptide Y release from the hypothalamus [11]. However, in line with the observation that isoforms of the leptin receptor are expressed in peripheral tissues such as skeletal muscle [12,13], more recent studies have shown that acute leptin administration alters both glucose and FA metabolism in skeletal muscle [14,15]. In isolated mouse soleus muscle incubated without insulin, short-term leptin treatment has been shown to increase FA oxidation and to decrease mTG synthesis [14]. In animals that have been administered a normal diet, short-term (<2 weeks) leptin treatment has also been reported to increase mTG hydrolysis in isolated rat soleus muscle incubated without insulin [16], to decrease FA transport into muscle giant sarcolemmal vesicles [17], and to reduce plasma membrane expression of FAT/CD36 and $FABP_{PM}$ in muscle [17]. These leptin-induced alterations in FA metabolism were associated with a decrease in mTG, suggesting that the insulin sensitizing effects of short-term leptin treatment on muscle glucose uptake [18] may be due in part to alterations in FA metabolism. In HF-fed rats, only the effects of short-term leptin treatment on basal FA metabolism have been studied in skeletal muscle and it was shown that the leptin-induced increase in FA oxidation and decrease in mTG synthesis observed in rats fed with normal rat chow was eliminated in rats fed a HF diet for 4 weeks [19]. These results suggested that with the accumulation of mTG induced by a HF diet, skeletal muscle develops not only insulin resistance but also leptin resistance [19]. Therefore, it is not known whether the insulin sensitizing effects of short-term leptin treatment on muscle glucose uptake observed in HF-fed rats would be associated with alterations in FA metabolism as shown in rats fed with normal rat chow [16,17].

Thus, the aims of this investigation were (1) to evaluate the effects of long-term (3 months) HF feeding on FA metabolism in muscle perfused under insulin-stimulated conditions and (2) to investigate the effects of short-term (10 days) leptin treatment on insulin sensitivity as it pertains to FA uptake, oxidation, and mTG synthesis in animals that have been administered an HF diet for 3 months.

## 2. Materials and methods

### 2.1. Animals

Seven-week-old male Wistar rats were randomly assigned to 1 of 4 groups whose initial body mass was not different ($P > .05$) between groups. One group was fed a control (CON) diet and 3 groups were fed a HF diet. The CON group (n = 8) was fed a low fat rat chow containing 4% fat, 24% protein, and 72% carbohydrate (Harlan Teklad, Madison, Wis), and given saline injections twice per day at 8:00 AM and 5:00 PM. The HF group (HF; n = 8) was fed a diet consisting of 65% fat, 22% protein, and 13% carbohydrate (Dyets Inc, Bethlehem, Pa), and was given saline injections twice per day. The HF-leptin (HF-LEP; n = 8) group was fed the HF diet and given leptin (5 mg/kg body weight; Amgen, Inc, Thousand Oaks, Calif) injections twice per day. The HF–pair fed (HF-P; n = 8) group was food restricted to the level of the HF-LEP group and given saline injections twice per day. This 10-day leptin regimen was used because it has been shown to be associated with similar serum leptin levels in CON and HF animals and physiologically elevated leptin levels in HF-LEP animals without a significant body weight discrepancy between the HF and HF-LEP groups [37]. Food intake of all groups was measured daily for the 10-day experimental period. To accomplish pair feeding, the HF-P animals received each day the same amount of HF food as what the HF-LEP animals had consumed the previous day. All animals had ad libitum access to water and were housed on a 12-hour light-dark cycle. Ethical approval for the study was granted from the Institutional Animal Care and Use Committee at the University of Southern California.

### 2.2. Hindlimb perfusions

On the day of the experiment, animals were anesthetized with an intraperitoneal injection of ketamine/xylazine (80 mg and 12 mg/kg body weight, respectively). Hindlimbs were surgically isolated as previously performed in our laboratory and described in detail [20,21]. Before the perfusion, catheters were inserted and 150 IU heparin was administered to the inferior vena cava. Rats were euthanized immediately before the insertion of the catheters by an intracardial injection of pentobarbital sodium (0.4 mg/g body weight). Immediately after the insertion of the catheters, 25 mL of perfusate was passed through the circulatory system and discarded to reduce possible effects of added heparin on plasma FA availability due to lipoprotein lipase activity. Hindlimbs were perfused for 20 minutes with Krebs-Henseleit solution, 1- to 2-day-old washed bovine erythrocytes (hematocrit 28%) containing 3.5% bovine serum albumin (Cohn

fraction V; Sigma, St Louis, Mo), [1-$^{14}$C]palmitate (ICN Pharmaceuticals, Costa Mesa, Calif), 600 $\mu$mol/L palmitate, 9 mmol/L glucose, and 100 $\mu$U/mL insulin. We chose this insulin concentration rather than a supraphysiological insulin concentration such as 1000 $\mu$U/mL because the goal of our study was to determine whether short-term leptin treatment affects insulin sensitivity rather than insulin responsiveness. The perfusate was continually gassed with 95% $O_2$ – 5% $CO_2$, and arterial samples were analyzed for hemoglobin and hematocrit content. At the end of the 20-minute equilibration period, the left iliac vessels were then tied off, and a clamp was fixed tightly around the proximal part of the leg to prevent bleeding. The right leg was then perfused at rest for 40 minutes at a perfusate flow of 5 mL/min (0.18 ± 0.01 mL · min$^{-1}$ · g$^{-1}$ perfused muscle for all groups; $P > .05$) and average pressure of 40.9 ± 4.4 mm Hg. Arterial and venous perfusate samples for the analysis of [$^{14}$C]FA and $^{14}CO_2$, as well as for FA and glucose concentrations, were taken at 10, 20, 30, and 40 minutes. Arterial and venous perfusate samples for determinations of $PCO_2$, $PO_2$, and pH were taken at 10 and 30 minutes and averaged 43.2 ± 2.5 and 173.2 ± 14.5 Torr and 7.3 ± 0.1 ($P > .05$), respectively, for all groups. At the end of the 40-minute perfusion period, the gastrocnemius muscle of the right leg was freeze-clamped in situ with aluminum clamps precooled in liquid nitrogen, taken out and stored in liquid nitrogen within 15 to 30 seconds.

### 2.3. Blood and perfusate analyses

Plasma FA levels were measured enzymatically with a commercial kit (NEFA C, Wako Chemicals, Richmond, Va) whereas glucose concentration was determined using the glucose oxidase method (YSI, Yellow Springs, Ohio). Perfusate $PCO_2$, $PO_2$, and pH were measured using an ABL5 analyzer (Radiometer America, Westlake, Ohio), and the collection of $^{14}CO_2$ liberated from the perfusate was performed as previously described [20,21]. The perfusate concentration of [$^{14}$C]palmitate was measured by liquid scintillation using a Hewlett Packard scintillation counter as previously described [21].

### 2.4. Muscle lipid analyses

Muscle triglyceride was determined as glycerol residues after extraction and separation of the muscle samples as previously described [21]. Briefly, lipids were extracted from powdered muscle samples by centrifugation in a chloroform-methanol (2:1) solution and magnesium chloride. The organic extract was evaporated and reconstituted in chloroform and silicic acid was added for the removal of phospholipids by centrifugation. The resulting supernatant was evaporated, saponified, and centrifuged. The final supernatant was analyzed for glycerol spectrophotometrically by the enzymatic glycerol kinase method (Sigma). To measure the incorporation of [$^{14}$C]palmitate into mTG, lipids from the extracted organic layer were separated by liquid chromatography and radioactivity in the mTG fraction was measured by liquid scintillation counting as previously described [21].

### 2.5. Calculations and statistical analyses

Palmitate kinetic parameters in blood and muscle were calculated as previously described [20,21]. Because there were no significant differences in values measured after 20, 30, and 40 minutes of perfusion, average values were used for each animal. Palmitate oxidation was corrected for label fixation using a previously determined acetate correction factor [22]. The arterial specific activity for palmitate did not vary over time and was not significantly different between groups averaging 50.1 ± 3.6, 46.3 ± 1.7, 47.1 ±1.2, and 51.2 ± 1.8 $\mu$Ci/mmol for the CON, HF, HF-LEP, and HF-P groups, respectively ($P > .05$). Statistical significance was determined using analysis of

Table 1
Effect of short-term leptin treatment or pair feeding on animal and perfusion characteristics in perfused hindlimb of HF-fed rats

| | CON | HF | HF-LEP | HF-P |
|---|---|---|---|---|
| *Animal characteristics* | | | | |
| Energy intake (kJ/d) | 326.1 ± 28.5 | 354.6 ± 9.6* | 290.1 ± 17.6** | 292.2 ± 8.4** |
| Body mass (g)—before 3-month diet | 249.3 ± 1.5 | 250.3 ± 2.8 | 253.0 ± 1.4 | 245.3 ± 3.8 |
| Body mass (g)—treatment day 0 | 474.9 ± 11.6*** | 495.3 ± 15.7*** | 492.2 ± 14.4*** | 450.2 ± 12.6*** |
| Body mass (g)—treatment day 10 | 469.7 ± 19.4*** | 497.7 ± 21.4*** | 484.2 ± 9.6*** | 439.2 ± 6.0*,*** |
| *Perfusate characteristics* | | | | |
| Glucose concentration (mmol/L) | 9.1 ± 0.3 | 8.7 ± 0.1 | 8.9 ± 0.2 | 9.0 ± 0.5 |
| Palmitate concentration ($\mu$mol/L) | 590.2 ± 28.8 | 623.0 ± 16.3 | 615.9 ± 15.2 | 591.3 ± 17.1 |
| Palmitate delivery (nmol · min$^{-1}$ · g$^{-1}$) | 78.4 ± 4.2 | 77.9 ± 3.0 | 79.3 ± 2.3 | 78.9 ± 1.7 |
| Oxygen uptake ($\mu$mol · h$^{-1}$ · g$^{-1}$) | 22.6 ± 2.2 | 21.2 ± 1.5 | 21.7 ± 1.2 | 19.7 ± 1.7 |

Values are means ± SE. CON indicates control given saline injection; HF, HF diet given saline injection; HF-LEP, HF diet given leptin injection; HF-P, HF diet–pair feeding given saline injection.

* $P < .05$, significantly different from all other groups.

** $P < .05$, significantly different from CON and HF.

*** $P < .05$, significantly different from respective group before 3-month diet.

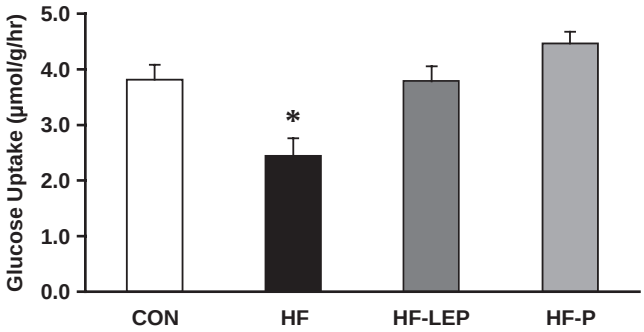


Fig. 1. Effect of short-term leptin treatment or pair feeding on glucose uptake by perfused hindlimbs. Values are means ± SE. Because there was no significant difference in values measured after 20, 30 and 40 minutes of perfusion, average values were used for each animal. CON indicates control given saline injection; HF, HF diet given saline injection; HF-LEP, HF diet given leptin injection; HF-P, HF diet–pair feeding given saline injection; asterisk, $P < .05$, significantly different from all other groups.

variance (Statistica, Tulsa, Okla) with Newman-Keul test for post hoc comparisons where appropriate. Pearson product-moment correlations were computed when appli-

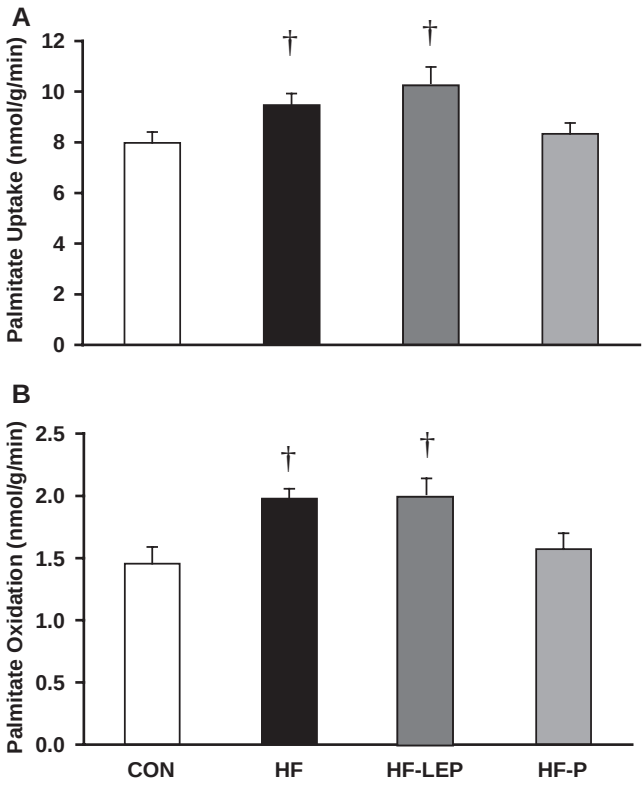


Fig. 2. Effect of short-term leptin treatment or pair feeding on palmitate uptake (A) and oxidation (B) by perfused hindlimbs. Values are means ± SE. Because there was no significant difference in values measured after 20, 30 and 40 minutes of perfusion, average values were used for each animal. Palmitate oxidation was corrected for label fixation, as described in Materials and methods. Dagger indicates $P < .05$, significantly different from CON and HF-P.

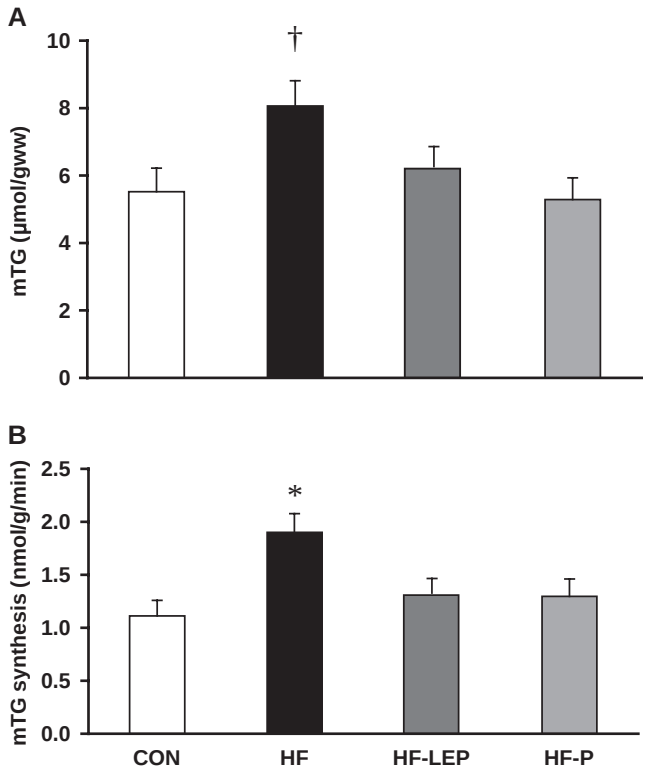


Fig. 3. Effect of short-term leptin treatment or pair feeding on mTG level (A) and mTG synthesis (B) in perfused hindlimbs. Values are means ± SE. Dagger indicates $P < .05$, significantly different from CON and HF-P; asterisk, $P < .05$, significantly different from all other groups.

cable. In all instances, an $\alpha$ of .05 was used to determine significance.

## 3. Results

### 3.1. Animal and perfusion characteristics

HF animals recorded a greater energy expenditure than all other groups, whereas energy expenditure was lower in the HF-LEP and HF-P groups relative to HF and CON animals

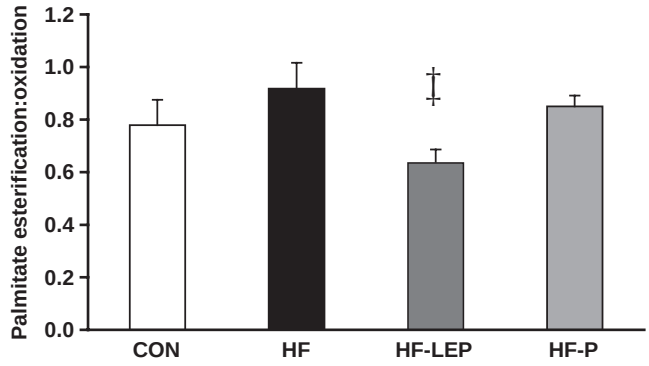


Fig. 4. Effect of short-term leptin treatment or pair feeding on the palmitate esterification to oxidation ratio in perfused hindlimbs. Values are means ± SE. Double dagger indicates $P < .05$, significantly different from HF and HF-P.

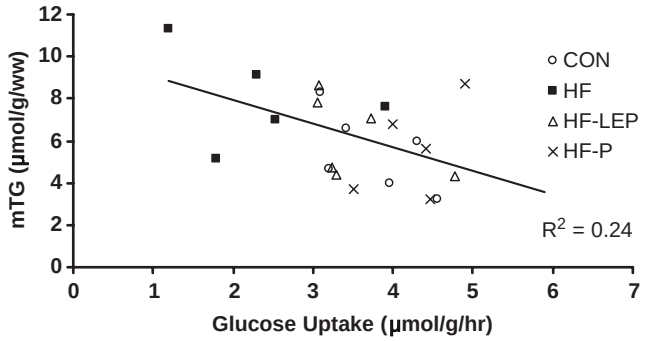


Fig. 5. Correlation between insulin-stimulated glucose uptake and mTG concentration in perfused hindlimbs ($y = -1.23x + 10.7$; $R^2 = -0.24$, $P = .021$).

(Table 1). After the 3 months of dietary manipulation, body mass was not significantly different between groups ($P > .05$) and averaged 474.9 ± 11.6 g in the CON group (n = 8) and 479.2 ± 14.2 g in the HF-fed groups (n = 24) (Table 1). Although the 10-day leptin treatment and pair-feeding regimen decreased body mass by 1.6% to 2.4%, these decreases were not significant ($P > .05$). However, at the end of the 10-day treatment period, body mass in the HF-P group was lower than in any of the other groups ($P < .05$). Plasma glucose ($P > .05$) and palmitate concentration ($P > .05$), palmitate delivery ($P > .05$), and oxygen uptake ($P > .05$) by muscle were not different between groups (Table 1). Three months of HF feeding led to a 40% decrease in insulin-stimulated glucose uptake that was restored by pair feeding with no additional effect of leptin (Fig. 1).

### *3.2. Palmitate metabolism under insulin-stimulated conditions*

Three months of HF feeding increased palmitate uptake by the hindlimb ($P < .05$, Fig. 2A) and this was accompanied by a 39% increase in palmitate oxidation ($P < .05$, Fig. 2B). When compared with the HF-P group, leptin treatment significantly increased both palmitate uptake ($P < .05$, Fig. 2A) and oxidation ($P < .05$, Fig. 2B) by 24% to 34%. Muscle triglyceride concentration in mixed gastrocnemius muscle was significantly greater in HF than in CON ($P < .05$, Fig. 3A) but was not significantly different from CON in HF-LEP and HF-P ($P > .05$). The rate of mTG synthesis was 43% to 70% higher ($P < .05$) in HF animals than in any other group (Fig. 3B). The ratio of palmitate esterification to oxidation was not significantly changed in HF or HF-P when compared with CON but was decreased by 30% in HF-LEP when compared with HF ($P < .05$, Fig. 4). Insulin-stimulated glucose uptake was found to correlate negatively with mTG concentration ($y = -1.23x + 10.7$; $R^2 = -0.24$, $P = .021$, Fig. 5).

## 4. Discussion

Our results show that muscle FA metabolism under insulin-stimulated conditions is altered by 3 months of HF feeding and is accompanied by resistance to the antioxidative actions of insulin but not to the lipogenic actions of insulin as shown by the high rate of FA oxidation and mTG synthesis in HF-fed rats. Our results also show that short-term leptin treatment in HF-fed rats increases FA uptake and oxidation independently of the leptin-induced food restriction and is accompanied by a reduction in the FA esterification-oxidation ratio which favors oxidative over nonoxidative FA disposal.

In agreement with results obtained after 3 weeks of HF feeding [7], our data show that FA oxidation under insulin-stimulated conditions is elevated in rats fed a HF diet for a prolonged duration. This is in agreement with data showing that muscle oxidative capacity as assessed by hydroxyacyl-CoA dehydrogenase activity [7] and by palmitoyl-CoA generated mitochondrial respiration [23] is increased by as little as 2 to 3 weeks of HF feeding. Because insulin is known to decrease FA oxidation in muscle [24,25], the high rate of FA oxidation in muscle of HF-fed rats indicates that resistance to the actions of insulin is not limited to glucose uptake but extends to the antioxidative actions of insulin. The mechanism behind this high rate of FA oxidation under insulin-stimulated conditions is not clear. It has been shown that the antioxidative effects of insulin on muscle FA metabolism may occur via the phosphatidylinositol 3-kinase cascade [24]. Because phosphatidylinositol 3-kinase and Akt/protein kinase B activity in response to insulin treatment has been shown to decrease with HF feeding [18,26,27], the high rate of FA oxidation in HF-fed rats may be due to a defect in early insulin signaling. Conversely, because long-term HF feeding is also associated with a decrease in insulin-stimulated glucose uptake ([18], Fig. 1), the increase in FA oxidation could be due indirectly to the reduced availability of glucose for oxidation. This would be expected to increase FA oxidation because the metabolic rate is unchanged between conditions as indicated by similar oxygen uptake during the perfusion (Table 1). Compared with data obtained after 3 weeks of HF feeding [7], the present results also show that long-term exposure to a HF diet is associated with further alterations in muscle FA metabolism, namely, an increase in FA uptake and mTG synthesis under insulin-stimulated conditions. Given that insulin in the presence of glucose has been reported to increase muscle FA uptake and mTG synthesis [24,25] and that FAT/CD36 messenger RNA expression has been shown to increase with HF feeding [28], our data show that resistance to the actions of insulin induced by 3 months of HF feeding does not extend to the FA transport system or the mTG synthesis pathway.

Leptin is known to decrease food intake [11] and our data show that 10 days of leptin treatment was associated with an 18% decrease in energy expenditure when compared with HF-fed rats. Because food restriction has been shown to affect glucose and FA metabolism in as little as 5 days [29,30], we included a HF group fed the same amount of energy as the HF-LEP group (HF-P). Thus, when looking at the effects of leptin independently of the effects of food

restriction, comparisons between the HF-LEP and HF-P groups are more appropriate. With this in mind, our data show that 10 days of leptin treatment in rats fed a HF diet for 3 months are associated with metabolic alterations beyond those observed with pair feeding alone and these alterations include an increase in insulin-stimulated FA uptake and oxidation but no change in mTG synthesis or glucose uptake. The effect of short- or long-term leptin treatment on basal FA uptake in muscle is controversial with a decrease being reported in giant sarcolemmal vesicles isolated from control rats treated with leptin for 2 weeks [17], but no effect being reported in isolated soleus muscle of control or HF-fed rats incubated acutely with leptin but no insulin [31]. Our data show that pair feeding alone in HF-fed rats brings back the rate of FA uptake under insulin-stimulated conditions to the level measured in CON and that short-term leptin treatment in HF-fed rats increases the capacity of the muscle to take up FA when compared with HF-P rats. Because HF feeding has been shown to be associated with leptin resistance [19], the high rate of FA uptake by HF-LEP animals might be representative of resistance to the possible down-regulatory actions of leptin on FA uptake [17]. However, acute and chronic leptin treatment has been shown to activate AMP-activated protein kinase (AMPK) [32,33] and we have shown that perfusion of rat hindlimbs with the AMPK activator AICAR is associated with an increase in FA uptake at rest [34]. Similarly, short-term leptin treatment has been shown to stimulate the activity of ERK2 in mouse skeletal muscle [35] and we have shown that muscle FA uptake is regulated via the ERK1/2 pathway under some conditions because inhibition of ERK1/2 activation abolished the contraction-induced increase in FA uptake [36]. Consistent with these previously published data, our results might then suggest that short-term leptin treatment in HF-fed rats elicits an increase in FA uptake via activation of AMPK or ERK1/2 signaling.

When compared with the HF-P group, our data also show that short-term leptin treatment was associated with an increase in FA oxidation under insulin-stimulated conditions. These results agree with those of Muoio et al [24] obtained in lean *ob/ob* mice and which show that leptin prevents the insulin-induced decrease in FA oxidation. The cellular mechanisms regulating the high rate of FA oxidation with leptin treatment are not clear. Short-term leptin treatment has been shown to increase basal FA oxidation in isolated soleus muscle of lean rodents [14,19] but not in soleus muscle of rats fed a HF diet for 4 weeks [19], suggesting that HF feeding might lead to leptin resistance. In contrast to these results, the high rate of FA oxidation in HF-LEP when compared to HF-P suggests that leptin sensitivity is maintained when HF-fed rats are given long-term treatment with leptin and that the effects of leptin on FA oxidation prevailed over the antioxidative effects of insulin.

We observed that although short-term leptin treatment was accompanied by a decrease in mTG synthesis when compared with the HF group, it did not decrease the mTG synthesis rate beyond that of HF-P indicating that the decrease in esterification was due primarily to the reduction in energy intake that accompanies short-term leptin treatment. These results agree with data showing that short-term leptin treatment does not affect mTG synthesis in HF-fed rats [31]. Thus, without changing the rate of mTG synthesis, short-term leptin treatment, but not pair feeding alone, was associated with a change in cellular FA disposal that favored oxidative FA disposal as shown by the significant decrease in the FA esterification-oxidation ratio.

Short-term leptin treatment restored insulin-stimulated glucose uptake to rates observed in HF-P and CON. Because insulin-stimulated glucose uptake was not different between HF-LEP and HF-P, our data suggest that pair feeding alone was sufficient to improve insulin action at the glucose uptake step. Our results obtained with a submaximal insulin concentration (100 $\mu$U/mL) are in contrast to data obtained with supraphysiological levels of insulin (500 $\mu$U/mL or 10 mU/mL) [37,38], and which showed that short-term leptin treatment increased insulin-stimulated glucose uptake more effectively than pair feeding alone. However, it has been reported that although 5 days of food restriction (75% of control energy intake) was associated with an increase in glucose uptake in epitrochlearis muscle incubated with a submaximal insulin concentration, glucose transport at a maximally effective insulin concentration was not altered [30]. Thus, our data are consistent with those of others [30,38] and show that although brief food restriction increases insulin sensitivity for glucose uptake, the effects of food restriction on insulin responsiveness may require a longer period of food restriction. As mentioned previously, recent studies have reported that short-term leptin treatment reverses the HF diet–induced decrease in early insulin signaling [18,38] and this was associated with an increase in plasma membrane GLUT4 levels in muscle [18,38] providing a mechanism for the reported increase in glucose uptake in HF-LEP animals.

In conclusion, we report that 3 months of HF feeding results in a decrease in glucose uptake and an increase in FA uptake and oxidation, and in mTG synthesis in muscle perfused under insulin-stimulated conditions. Short-term leptin treatment, independently of the leptin-induced reduction in energy intake, increased FA uptake and oxidation but did not affect glucose uptake or mTG synthesis under insulin-stimulated conditions. As shown by the decrease in FA esterification-oxidation ratio, these results suggest that short-term leptin treatment improves cellular FA disposal by increasing FA oxidation.

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