Chronic Leptin Administration Increases Insulin-Stimulated Skeletal Muscle Glucose Uptake and Transport

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Leptin, the product of the *ob* gene, has been shown to reduce fat mass, food intake, hyperglycemia, and hyperinsulinemia and to increase whole-body glucose disposal. However, it is unknown if leptin improves insulin action in skeletal muscle. Therefore, the purpose of this investigation was to determine if chronic leptin administration increases insulin-stimulated skeletal muscle glucose uptake and transport. Sixty-nine female Sprague-Dawley rats (240 to 250 g) were randomly assigned to one of three groups: (1) control, (2) pair-fed, and (3) leptin. All animals were subcutaneously implanted with miniosmotic pumps that delivered 0.5 mg leptin/kg/d to the leptin animals and vehicle to the control and pair-fed animals for 14 days. Following this 14-day period, all animals were subjected to hindlimb perfusion to determine the rates of skeletal muscle glucose uptake and 3-*O*-methyl-D-glucose (3-MG) transport under basal, submaximal (500 μU/mL), and maximal (10,000 μU/mL) insulin concentrations. Chronic leptin treatment significantly increased ($P < .05$) the rate of glucose uptake across the hindlimb by 27%, 32%, and 47% under basal, submaximal, and maximal insulin, respectively, compared with the control and pair-fed condition. However, when the submaximal rate of glucose uptake was expressed as a percentage of maximal insulin-stimulated glucose uptake, no differences existed among the groups, indicating that leptin treatment does not increase insulin sensitivity. Rates of 3-MG transport in the soleus, plantaris, and white and red portions of the gastrocnemius (WG and RG) were significantly increased ($P < .05$) in leptin animals under all perfusion conditions. 3-MG transport was not different between control and pair-fed animals. Collectively, these findings suggest that improvements in insulin-stimulated skeletal muscle glucose uptake and transport following chronic leptin treatment result from increased insulin responsiveness.

IT IS WELL DOCUMENTED that obesity, especially extensive visceral adipose deposition, is associated with resistance to insulin-stimulated glucose uptake and may ultimately lead to the development of non–insulin-dependent diabetes.[1] Of particular concern is that individuals who exhibit compensatory hyperinsulinemia for the maintenance of glucose homeostasis may be predisposed to develop a number of metabolic abnormalities including hypertriglyceridemia, reduced high-density lipoprotein, elevated apolipoprotein B, and hypertension, which are said to comprise syndrome X or "insulin resistance syndrome."[2] It would therefore appear that interventions that reduce obesity might improve insulin-stimulated glucose uptake and ameliorate the associated metabolic complications.

To this end, leptin, the product of the *ob* gene,[3] has recently received considerable attention because its administration has been shown to reduce fat mass, food intake, hyperglycemia, and hyperinsulinemia.[4,5] It is believed that leptin exerts its primary effect by acting on receptors in the hypothalamus and inhibiting the release of neuropeptide Y.[6] However, leptin has been shown to exert effects in tissues other than the hypothalamus.[7,8] Thus, the possibility exists that leptin may have actions that are ubiquitous throughout the whole body. Of particular interest, Barzilai et al[9] found that following 8 days of leptin treatment in Sprague-Dawley rats, whole-body glucose uptake as assessed by the euglycemic clamp technique was increased 52% compared with control animals. Since over 80% of a glucose load is disposed of by skeletal muscle,[10] it is possible that leptin may be capable of acting on skeletal muscle to improve insulin action. This hypothesis is supported by the observation that leptin receptor isoforms are expressed in tissues other than the hypothalamus and insulin action is improved in these tissues following leptin treatment.[11-13] Therefore, one purpose of this investigation was to determine directly whether the process of insulin-stimulated glucose uptake and transport is improved in normal rat skeletal muscle following chronic leptin administration as assessed during hindlimb perfusion.

Additionally, since the hindlimb perfusion procedure was used in this investigation, the effect of chronic leptin administration on insulin-stimulated glucose transport in individual fiber types could be assessed. Skeletal muscle in the rat can be classified by metabolic and contractile characteristics into three basic fiber types: slow-twitch red, fast-twitch red, and fast-twitch white.[14,15] These fiber types differ in insulin sensitivity and responsiveness, largely due to differences in the GLUT4 protein concentration and oxidative capacity.[16,17] Thus, a second purpose of this investigation was to determine if chronic leptin administration differentially affects insulin action in the three basic skeletal muscle fiber types.

MATERIALS AND METHODS

Experimental Animals

Sixty-nine female Sprague-Dawley rats weighing approximately 240 to 250 g were obtained from B & K Universal (Fremont, CA) and randomly assigned to one of three groups: (1) control (n = 22), (2) pair-fed (n = 23), and (3) leptin (n = 24). Upon arrival, the rats were housed three per cage in a temperature-controlled animal room (21°C) maintained on a 12-hour light-dark cycle. The rats were provided standard rat chow (BeeKay Feed; B & K Universal) and water ad libitum. Two weeks prior to hindlimb perfusion, all animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (3 mg/100 g body weight) and subcutaneously implanted on the back, slightly posterior to the scapulae, with Alzet miniosmotic pumps (2ML2; Alza Scientific Products, Palo Alto, CA). The miniosmotic pumps have a reservoir volume of 2 mL and a nominal pumping rate of

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5 μL/h that can be maintained for a minimum of 14 days. Control and pair-fed animals were implanted with miniosmotic pumps filled with phosphate-buffered saline (vehicle), while leptin animals were implanted with osmotic pumps filled with 1 mg/mL recombinant murine leptin (Amgen, Thousand Oaks, CA). Following insertion of the osmotic pumps, the animals were housed individually.

Leptin administration has previously been found to decrease daily food intake by approximately 30%.[4] To account for this difference in daily food intake and the subsequent reduction in body weight compared with the control animals, pair-fed animals had food intake restricted to match that which the leptin group spontaneously consumed. Pair feeding was accomplished by assigning one pair-fed rat to each leptin-treated rat. The amount of rodent diet provided to each pair-fed rat was equivalent to the amount eaten during the previous 24 hours by the matched leptin-treated rat. Control animals were provided standard rat chow and water ad libitum during the 14-day experimental period. Body weight and food consumption were recorded daily for all rats.

All experimental procedures were approved by the Institutional Animal Care and Use Committee at California State University Northridge, and conformed with the guidelines for the use of laboratory animals published by the US Department of Health and Human Resources.

Surgical Preparation and Hindlimb Perfusion

Following 14 days of leptin or vehicle delivery, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (6.5 mg/100 g body weight). The surgical technique to prepare the rats for perfusion and the perfusion apparatus were similar to those described previously by Ruderman et al[18] and modified by Ivy et al.[19] The right iliac artery was catheterized to the tip of the femoral artery to limit perfusate flow to the right hindlimb. Catheterization of the lower abdominal vena cava to the tip of the iliac vein permitted the collection of effluent perfusate.

Immediately after catheterization of the vessels, the rats were killed via an intracardiac injection of pentobarbital while the hindlimbs were washed out with 10 mL Krebs-Heinseleit buffer (KHB). The catheters were then placed in line with a nonrecirculating perfusion system, and the hindlimb was allowed to stabilize during a 5-minute washout period. The perfusate was continuously gassed with a mixture of 95% O_2–5% CO_2 and warmed to 37°C. The perfusate flow rate was set at 5 mL/min during the 5-minute stabilization and the subsequent perfusion, during which the rates of muscle glucose uptake and glucose transport were determined.

Perfusions were performed in the absence of insulin (n = 8 per group) and in the presence of a submaximal (500 μU/mL, n = 7 to 8 per group) and maximal (10,000 μU/mL, n = 7 to 8 per group) insulin concentration for the three experimental groups. The basic perfusate medium consisted of 30% washed time-expired human erythrocytes (HemaCare, Van Nuys, CA), KHB (pH 7.4), 4% dialyzed bovine serum albumin (Fraction V; Fisher Scientific, Fair Lawn, NJ), and 0.2 mmol/L pyruvate. Over the first 20 minutes, 8 mmol/L glucose was present in the perfusate, and it was during this period that glucose uptake was measured across the hindlimb. Subsequent to the determination of glucose uptake, the hindlimb was washed out with glucose-free perfusate for 1 minute in preparation for the measurement of glucose transport. Glucose transport was measured over an 8-minute period using an 8-mmol/L concentration of the nonmetabolizable glucose analog 3-*O*-methyl-D-glucose ([3-MG] 32 μCi 3-[^{3}H]MG/mmol) and 2 mmol/L mannitol (60 μCi D-[1-^{14}C]mannitol/mmol). Immediately at the end of the transport period, the soleus, plantaris, and red (RG) and white (WG) portions of the gastrocnemius were excised from the right leg, blotted on gauze dampened in cold KHB, and clamp-frozen in tongs cooled in liquid N_2. The muscles were stored at −80°C until analyzed for 3-MG transport.

Measurement of Glucose Uptake

Glucose uptake was determined over a 20-minute nonrecirculating perfusion by collecting arterial perfusate samples before perfusion and collecting the total venous effluent. Well-mixed aliquots of the arterial perfusate and venous effluent were analyzed for the glucose concentration by a glucose oxidase method on a model 2300 STAT Plus glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Muscle glucose uptake, expressed in micromoles per gram per hour, was calculated from the arteriovenous difference, the perfusate flow rate, and the weight of the muscle perfused. The weight of the perfused muscle was determined by dissection of the rat hindlimb.[20]

Determination of 3-MG Transport

Muscle samples were weighed, homogenized in 1 mL 10% trichloroacetic acid (TCA) at 4°C, and centrifuged in a microcentrifuge (Fisher Scientific, Houston, TX) for 10 minutes. Duplicate 300-μL samples of the supernatant were transferred to 7-mL scintillation vials containing 6 mL Bio-Safe II scintillation counting cocktail (Research Products International, Mount Prospect, IL) and vortexed. For determination of perfusate specific activity, 200 μL arterial perfusate was added to 800 μL 10% TCA and treated the same way as the muscle homogenates. The samples were counted for radioactivity in a LS 1801 liquid scintillation spectrophotometer (Beckman Instruments, Fullerton, CA) set for simultaneous counting of ^{14}C/^{3}H. The accumulation of intracellular 3-[^{3}H]MG, which is indicative of muscle glucose transport, was calculated by subtracting the concentration of 3-[^{3}H]MG in the extracellular space from the total muscle 3-[^{3}H]MG concentration. 3-[^{3}H]MG in the extracellular space was quantified by measuring the concentration of [^{14}C]mannitol in the homogenate.

Statistical Analysis

A one-way ANOVA was used on all variables to determine whether significant differences existed between the control, pair-fed, and leptin groups. When a significant F ratio was obtained, a Fisher's protected least-significant difference post hoc test was used to identify statistically significant differences ($P < .05$) among the means.

RESULTS

Body Weight, Muscle Weight, and Food Consumption

The body weight of the animals was not significantly different among the groups before implantation of the osmotic pumps. Over the first 3 days of the experimental period, no difference in body weight was observed among groups. Beginning at day 4 and continuing to the end of the experimental period, leptin and pair-fed animals exhibited a significantly lower body weight compared with the control animals. No differences in body weight existed between leptin and pair-fed animals until day 14 of the experimental period (Fig 1).

Following the 14-day treatment period, the soleus of control animals was significantly heavier than that of leptin animals. Soleus weight did not differ between control and pair-fed animals or pair-fed and leptin animals. The plantaris of pair-fed and leptin animals was similar in weight but significantly lighter compared with control animals (Table 1).

Food consumption was found to be significantly reduced by about 31% in leptin animals compared with control animals on the day immediately following implantation of the miniosmotic

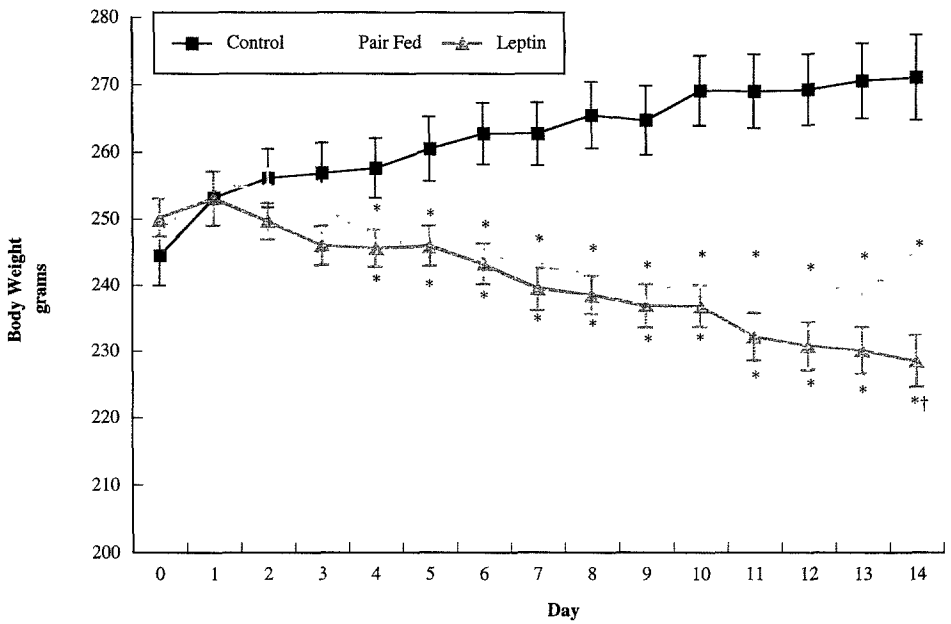

Fig 1. Body weight of control (n = 22), pair-fed (n = 23), and leptin-treated (n = 24) female Sprague-Dawley rats over the 14-day experimental period. Values are the mean ± SE. *$P < .05$ *v* control. †$P < .05$ *v* pair-fed.

pump, and remained at this significantly reduced level throughout the 14-day experimental period. Daily food consumption of the pair-fed animals was virtually identical to that of the leptin animals and was also significantly lower than that of the control group at all time points during the 14-day treatment period (Fig 2).

Hindlimb Glucose Uptake

Administration of leptin for 14 days significantly elevated the rate of hindlimb glucose uptake. Compared with control and pair-fed animals, hindlimb glucose uptake was increased in leptin animals by about 27%, 32%, and 47% under basal, submaximal, and maximal insulin stimulation, respectively (Fig 3).

However, glucose uptake expressed as a percentage of maximal insulin-stimulated glucose uptake at the submaximal insulin concentration was not significantly different among groups. The percent of maximal insulin-stimulated glucose uptake was 66%, 78%, and 67% for control, pair-fed, and leptin animals, respectively, in the presence of a submaximal insulin concentration. The percent of maximal insulin-stimulated glucose uptake in the absence of insulin (basal) was 35%, 44%, and 35% for control, pair-fed, and leptin animals, respectively.

3-MG Transport

The rate of glucose transport was determined in the soleus, plantaris, WG, and RG under basal, submaximal, and maximal insulin-stimulated conditions using the glucose analog 3-MG. 3-MG is carried by the glucose transporter and is not phosphorylated, which results in its intracellular accumulation representing the glucose transport process independent of intracellular disposal. Following 14 days of leptin treatment, the rate of 3-MG transport in the soleus, plantaris, WG, and RG of leptin animals was significantly increased under submaximal and maximal insulin stimulation as compared with control and pair-fed animals. Under basal conditions, the rate of 3-MG transport was significantly greater in the soleus, plantaris, and RG of leptin animals compared with control and pair-fed animals. No differences in the rate of glucose transport in the collected muscles were observed between control and pair-fed animals under the three perfusion conditions (Figs 4 to 6).

Table 1. Soleus and Plantaris Weight (mg) in Female Sprague-Dawley Rats Following a 14-Day Treatment Period

Muscle	Control (n = 22)	Pair-Fed (n = 23)	Leptin (n = 24)
Soleus	106.9 ± 2.7	102.7 ± 2.5	96.3 ± 1.7*
Plantaris	258.0 ± 5.5	241.9 ± 4.2*	232.9 ± 5.5*

NOTE. Values are the mean ± SE.
*$P < .05$ *v* control.

DISCUSSION

Chronic leptin administration has been shown to reduce body weight, visceral fat deposition, food intake, hyperinsulinemia, and hyperglycemia and to increase whole-body glucose disposal.[4,5,9] However, it is unknown if chronic leptin administration has an effect on carbohydrate metabolism in skeletal muscle. Thus, the aim of the present investigation was to determine if chronic leptin administration alters insulin-stimulated skeletal muscle glucose uptake and transport as assessed during hindlimb perfusion.

Food consumption was found to be significantly reduced by about 31% in leptin animals compared with control animals. This observation is in excellent agreement with that of Chen et al.[4] who found leptin administration to reduce daily food intake by 30% in male Wistar rats. Also in agreement with previous investigations is our finding that chronic leptin administration reduced body weight compared with control animals.[21,22] An interesting difference between pair-fed and leptin animals was that leptin animals continued to lose weight linearly over the 14 days of treatment, but weight loss for pair-fed animals started to plateau at day 10 and was significantly different compared with

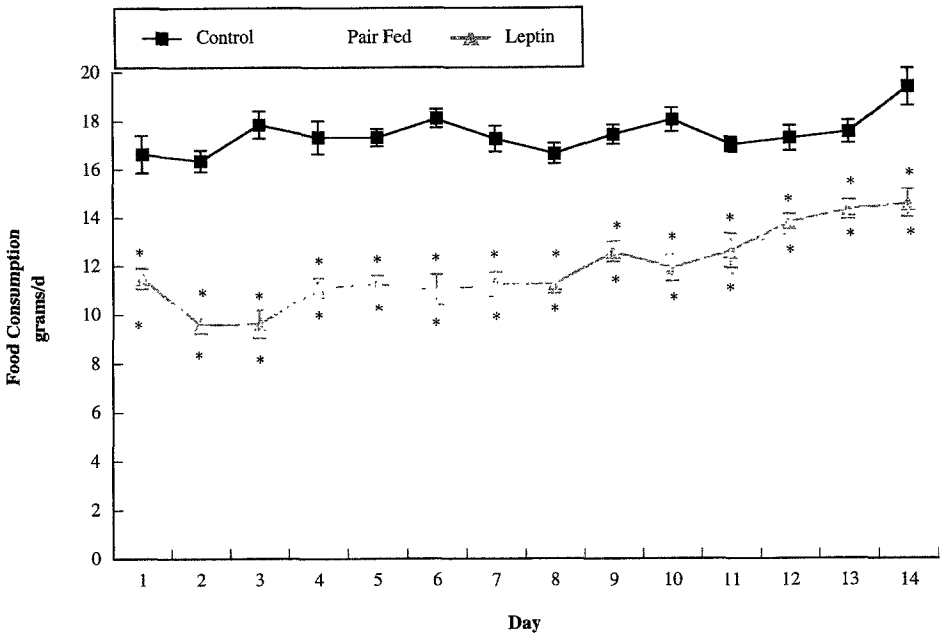

Fig 2. Daily food consumption of control (n = 22), pair-fed (n = 23), and leptin-treated (n = 24) female Sprague-Dawley rats over the 14-day experimental period. Values are the mean ± SE. *$P < .05$ *v* control.

leptin animals by day 14. This suggests that a change in food conversion efficiency and possibly the metabolic rate occurred in pair-fed animals but not in leptin animals. However, daily food consumption was not different between the leptin and pair-fed groups, which is of particular importance since it has been demonstrated that caloric intake can influence insulin action and membrane permeability to glucose in skeletal muscle.[23] Given that leptin and pair-fed animals consumed an identical amount of food throughout the experimental period, any differences in insulin-stimulated glucose uptake and transport that existed between the two groups can presumably be attributed to the effects of chronic leptin treatment.

In an extension to the findings of Barzilai et al,[9] who reported that chronic leptin administration increases whole-body glucose disposal, we observed that 14 days of leptin treatment significantly elevated skeletal muscle glucose uptake. Compared with control and pair-fed animals, hindlimb glucose uptake was increased in leptin animals in the absence of insulin, in the presence of a submaximal insulin concentration, and in the presence of a maximal insulin concentration. However, to calculate if insulin sensitivity was improved following leptin administration, glucose uptake was expressed as a percentage of maximal insulin-stimulated glucose uptake both at the submaximal insulin concentration and in the absence of insulin. It was observed that there were no significant differences in the percentage of maximal response among the experimental groups. Collectively, these observations suggest that the improvements in glucose uptake following 14 days of leptin treatment were a result of an improved insulin responsiveness as opposed to an improved insulin sensitivity. However, the possibility exists that these effects may not have occurred had the present investigation used an obese rodent model.

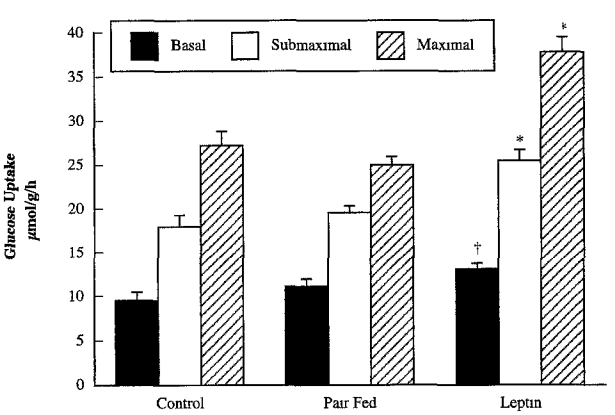

Fig 3. Glucose uptake in hindlimb of control (n = 22), pair-fed (n = 23), and leptin-treated (n = 24) rats during perfusion with 8 mmol/L glucose and basal (8 animals per group), submaximal (500 μU/mL, 7-8 animals per group), and maximal (10,000 μU/mL, 7-8 animals per group) insulin. Values are the mean ± SE. *$P < .05$ *v* control and pair-fed. †$P < .05$ *v* control.

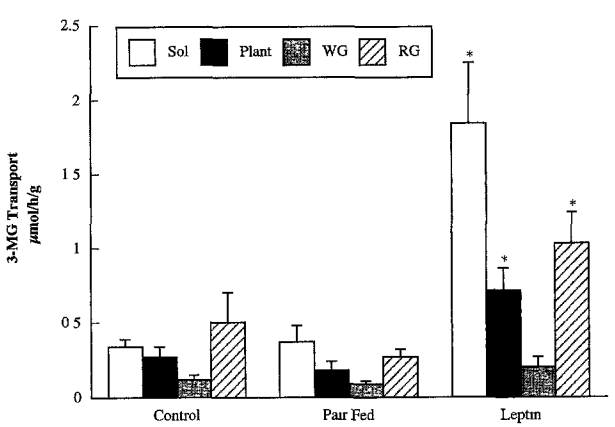

Fig 4. 3-MG transport under basal conditions in hindlimb muscles of control (n = 8), pair-fed (n = 8), and leptin (n = 8) animals. Sol, soleus; plant, plantaris. Values are the mean ± SE. *$P < .05$ *v* control and pair-fed.

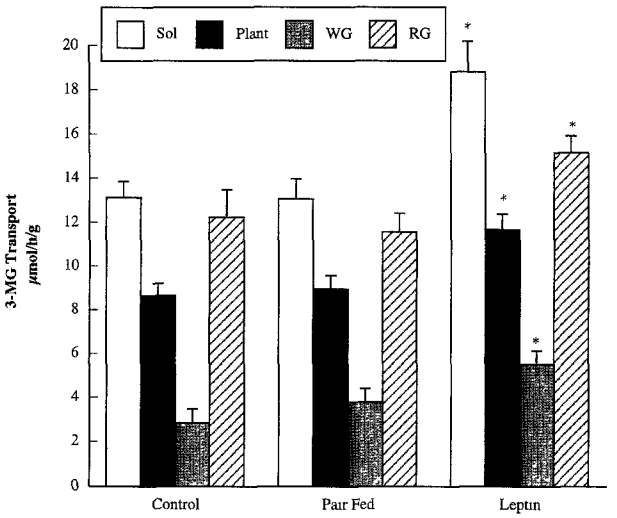

Fig 5. 3-MG transport in the presence of 500 μU/mL insulin in hindlimb muscles of control (n = 7), pair-fed (n = 8), and leptin (n = 8) animals. Sol, soleus; plant, plantaris. Values are the mean ± SE. *$P < .05$ *v* control and pair-fed.

Insulin sensitivity is a function of the ligand binding to its receptor, whereas insulin responsiveness is a measure of postreceptor effects.[24] Thus, since leptin did not change insulin sensitivity, it does not appear that chronic leptin treatment alters insulin binding to its receptor. Rather, it appears that the improvements in skeletal muscle glucose uptake observed in the present investigation were mediated at a postreceptor step. This may have been a result of chronic leptin treatment initiating direct effects in the skeletal muscle or secondary effects in response to a reduced visceral fat deposition.

The rat hindlimb is composed of muscles with different fiber types, which might have differentially contributed to the improvements in insulin responsiveness observed. Using the nonmetabolizable and nonphosphorylatable glucose analog 3-MG, we found that the rates of glucose transport under both submaximal and maximal insulin stimulation during hindlimb perfusion were significantly increased in all three skeletal muscle fiber types following 14 days of chronic leptin treatment. This is in contrast to acute (2 to 4 hours) leptin treatment, which does not affect insulin-stimulated skeletal muscle glucose transport.[25,26] To our knowledge, this is the first investigation to report that chronic leptin treatment increases the rate of insulin-stimulated skeletal muscle glucose transport. Moreover, another unique aspect of the present investigation is the utilization of the hindlimb perfusion technique to assess the effects of chronic leptin treatment on skeletal muscle metabolism. This procedure eliminates the influence of hormonal or systemic effects that normally exist in an intact animal model (ie, insulin or euglycemic clamp studies). Therefore, these observations would suggest that the improvements in insulin-stimulated glucose transport following chronic leptin administration were due to changes that occurred within the skeletal muscle.

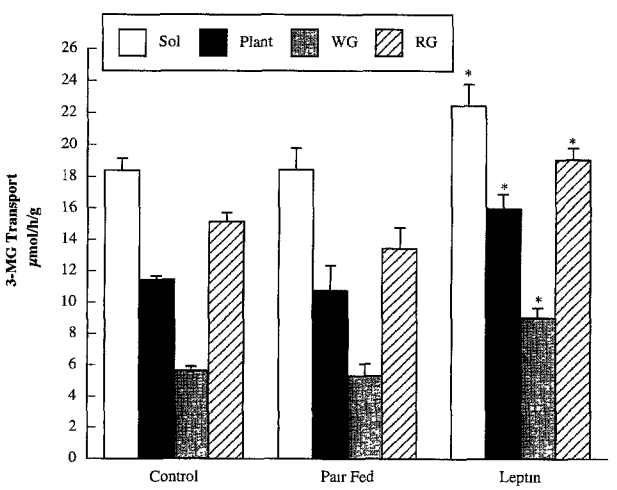

Fig 6. 3-MG transport in the presence of 10,000 μU/mL insulin in hindlimb muscles of control (n = 7), pair-fed (n = 7), and leptin (n = 8) animals. Sol, soleus; plant, plantaris. Values are the mean ± SE. *$P < .05$ *v* control and pair-fed.

It has been demonstrated that leptin administration will enhance phosphatidylinositol 3-kinase activity in response to insulin stimulation.[7] If the insulin signaling cascade is potentiated, the rate of insulin-stimulated glucose transport is increased. Chronic leptin treatment may elevate the skeletal muscle GLUT4 protein concentration. The rate of insulin-stimulated 3-MG transport has consistently been demonstrated to be directly related to the skeletal muscle GLUT4 protein concentration.[16,17,27] Finally, it is possible that the rate of insulin-stimulated skeletal muscle glucose transport following chronic leptin treatment may be elevated in response to an improved translocation of the glucose transporters or insertion into the plasma membrane due to alterations in membrane composition and/or fluidity.

However, it is plausible that the improvements in skeletal muscle insulin responsiveness following chronic leptin administration were due to peripheral adaptations occurring in the muscle as a secondary response to a reduction in visceral fat as opposed to leptin's exerting primary effects on the skeletal muscle. Chronic leptin administration has been demonstrated to reduce visceral fat stores, which in turn has been suggested to improve whole-body glucose disposal.[9] The manner by which a reduction in visceral fat deposition relates to an improved skeletal muscle insulin responsiveness is uncertain, but is believed to be partially accounted for by a reduced free fatty acid availability.[28]

While it is clear from the present investigation that chronic leptin treatment improves insulin-stimulated glucose uptake and transport in non–insulin-resistant skeletal muscle, it is not known whether these adaptations are a result of primary effects that occurred within the skeletal muscle following chronic leptin treatment or are secondary to a reduction in visceral fat deposition. It therefore appears that further investigation is warranted to identify the mechanism or mechanisms by which chronic leptin treatment initiates the improvements in skeletal muscle carbohydrate metabolism.

ACKNOWLEDGMENT

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