Chronic Leptin Treatment Normalizes Basal Glucose Transport in a Fiber Type-Specific Manner in High-Fat–Fed Rats

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The aim of this investigation was to determine if a high-fat diet impaired and subsequent leptin administration improved non–insulin-stimulated (basal) glucose transport in rodents. Twenty-four male Sprague Dawley rats were divided into 1 of 2 groups: (1) normal diet (control [CON], n = 8) or (2) high-fat diet (n = 16) and received standard rat chow or a high-fat diet, respectively, for 12 weeks. The high-fat diet animals were then further subdivided into high fat (HF) (n = 8) or high-fat-leptin (HF-LEP) (n = 8) groups. The HF-LEP animals were injected with leptin (10 mg leptin/kg/d), while the CON and HF animals received vehicle over a 12-day treatment period. Following the 12-day treatment period, all animals were subjected to hind limb perfusion to assess rates of basal skeletal muscle 3-O-methyl-D-glucose (3-MG) transport. Compared with the CON group, rates of 3-MG transport were reduced in the soleus (sol) and plantaris (plant) of the HF, but not the HF-LEP animals. Differences in skeletal muscle 3-MG transport could not be accounted for by an altered GLUT1 protein concentration. In contrast, a high-fat diet reduced and chronic leptin treatment normalized the skeletal muscle GLUT4 protein concentration. The results indicate that a high-fat diet reduces and subsequent leptin treatment improves basal skeletal muscle glucose transport in a fiber-type-specific manner, but these changes do not appear to be due to alterations in the GLUT1 protein concentration.

LEPTIN, THE PRODUCT of the *ob* gene,[1] has received a great deal of attention since its discovery in 1994, due to the ability of this 16-kd protein to reduce visceral adipose deposition.[2,3] It is believed that leptin exerts it primary effect by acting on receptors in the hypothalamus, possibly via inhibition of neuropeptide Y release.[4] However, leptin receptor isoforms have been found to be expressed in tissues other than the hypothalamus,[5-7] and that insulin action is improved in these tissues following leptin treatment.

The improvements in insulin-stimulated glucose disposal following chronic leptin administration were initially demonstrated by Barzilai et al.[8] These investigators reported that 8 days of leptin treatment increased whole body glucose uptake in Sprague Dawley rats as assessed by the euglycemic clamp technique. In an extension to these findings, we have reported that leptin administration increases insulin-stimulated skeletal muscle glucose uptake and 3-O-methyl-D-glucose (3-MG) transport in hind limbs of normal[9] and high-fat diet-induced insulin-resistant Sprague Dawley rats.[10] However, it is unknown if a high-fat diet and chronic leptin administration affects non–insulin-stimulated glucose transport in skeletal muscle from rodents provided a high-fat diet.

Therefore, the aims of the present investigation were to: (1) evaluate if a high-fat diet impairs non–insulin-stimulated (basal) skeletal muscle 3-MG glucose transport, (2) determine if chronic leptin administration affects basal 3-MG transport in skeletal muscle from high–fat-fed rats, and (3) assess if a high-fat diet and leptin treatment alters the content and basal distribution of the skeletal muscle glucose transporters, GLUT1 and GLUT4.

MATERIALS AND METHODS

Animals

Twenty-four male Sprague-Dawley rats approximately 6 weeks of age were obtained from B & K Universal Inc (Fremont, CA), housed 2 per cage and provided either a control diet (17% fat-derived calories, Dyets Inc, Bethlehem, PA, n = 8) or high-fat diet (59% fat-derived calories, Dyets Inc, n = 16) ad libitum for 3 months. The protein and carbohydrate contents, respectively, of the control diet were 20% and 63% and 15% and 26% in the high-fat diet.

Following this 3-month feeding period, high-fat–fed rats were then assigned to 1 of 2 treatment groups: high-fat diet (HF, n = 8) or high-fat diet, leptin-treated (HF-LEP, n = 8). Treatments were given for 12 days, during which time rats continued to consume the high-fat diet ad libitum. The HF-LEP group was injected subcutaneously (SC) twice daily with recombinant murine leptin (5 mg/kg/injection, 10 mg/kg/d total dose) provided by Amgen Inc (Thousand Oaks, CA). The HF group received twice daily SC injections of vehicle (phosphate-buffered saline, [PBS]). Control (CON) rats continued to consume the control diet ad libitum during the treatment periods and were injected twice daily SC with PBS. We have previously used this dosing regimen and found that serum leptin levels do not differ between CON and HF animals, but are significantly elevated in HF-LEP animals.[10]

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

Hind Limb Perfusions

Following the 12-day treatment period, animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (6.5 mg/100 g body weight) and surgically prepared for hind limb perfusion as described previously by Ruderman et al[11] and modified by Ivy et al.[12] After the surgical preparation, the quadriceps were excised from the left leg, frozen in liquid nitrogen, and stored at -80°C until analyzed for total GLUT4, total GLUT1, and intramuscular triglyceride (IMTG) concentration. The right iliac artery was then catheterized to the tip of the femoral artery to limit perfusate flow to the right hind limb.

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

Perfusions were performed in the absence of insulin for all experimental groups. The basic perfusate medium consisted of 30% washed time-expired human erythrocytes (HemaCare Corp, 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 5-minute washout, 8 mmol/L glucose was present in the perfusate. Subsequent to the 5-minute washout period, glucose transport was then measured over an 8-minute period using an 8 mmol/L concentration of the nonmetabolizable glucose analog 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 (Sol), plantaris (Plant), red (RG), and white (WG) portions of the gastrocnemius and quadriceps were excised from the right leg, clamp frozen in liquid N_2, and stored at -80°C until analyzed.

3-MG Transport

Muscle samples were weighed, homogenized in 1 mL of 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 of Bio-Safe II scintillation counting cocktail (Research Products International Corp, Mount Prospect, IL) and vortexed. For determination of perfusate specific activity, 200 μL of the arterial perfusate was added to 800 μL of 10% TCA and treated the same 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 ^{3}H/^{14}C. 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. The 3-[^{3}H]MG in the extracellular space was quantified by measuring the concentration of [^{14}C]mannitol in the homogenate.

Glucose Transporter Concentration

Total skeletal muscle GLUT4 and GLUT1 concentrations were determined by Western blotting. Portions of the freeze clamped muscles were weighed frozen and then homogenized in Hepes-EDTA-sucrose (HES) buffer (pH = 7.4). The protein concentration of the homogenate was determined by the Bradford method.[13] A 100-μL sample of the tissue homogenate was diluted 1:1 with Laemmli[14] sample buffer. An aliquot of the diluted homogenate sample containing 75 μg of protein was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) run under reducing conditions on a 12.5% resolving gel on a Mini-Protean II dual slab cell (Bio-Rad, Richmond, CA). Resolved proteins were transferred to polyvinylidene difluoride (PVDF) sheets (Bio-Rad, Richmond, CA) by the method of Towbin et al[15] using a Bio-Rad wet transfer unit. The membranes were incubated with an affinity purified polyclonal α GLUT4 (1:1,000) or α GLUT1 (1:1,000) (donated by Dr Samuel W. Cushman, National Institute of Diabetes and Digestive and Kidney Diseases [NIDDK], Bethesda, MD) followed by incubation with HRP-labeled Protein A (1:4,000) (Amersham Life Science, Arlington Heights, IL). Antibody binding was visualized using enhanced chemiluminescence autoradiography in accordance with the manufacturer's instructions (Amersham Life Science). Labeled bands were quantified by capturing images of the autoradiographs in a Macintosh G4 computer (Cupertino, CA). The captured images of the autoradiographs were produced by an image scanner (ScanJet 4C; Hewlett Packard, Palo Alto, CA) equipped with a transparency module. The captured images were digitized and imported into the public domain NIH image program (developed at the US National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). The density of the labeled bands was calculated, corrected for background activity, and expressed as a percentage of a standard (30 μg of heart homogenate protein) run on each gel.

Plasma membrane (PM) fractions were prepared from portions of the perfused quadriceps according to the procedure of Turcotte et al.[16] Briefly, a portion of the quadriceps was minced, diluted 1:7 in a 10 mmol/L Tris-15% sucrose solution (pH 7.5) that contained 0.1 mmol/L phenylmethylsulfonyl fluoride, 10 mmol/L EGTA, and 10 mg/mL trypsin inhibitor and homogenized with a PT 2100 Polytron homogenizer (Kinematica AG, Littau/Luzern, Switzerland). The homogenate was filtered and centrifuged at 100,000 $\times$ *g* for 1 hour using a Sorvall T-1250 rotor (Kendro Laboratory Products, Newton, CT). The pellet was resuspended in 10 mmol/L Tris-15% sucrose buffer, and a small aliquot from this resuspension was collected, retained for analysis, and will be referred to as the crude homogenate (CH). The remaining CH suspension was layered onto continuous sucrose gradients (35% to 70%) and centrifuged at 120,000 $\times$ *g* for 2 hours in a Sorvall Surespin 630/36 rotor. The PM layer was collected, washed in 10 mmol/L Tris buffer and centrifuged for 1 hour at 100,000 $\times$ *g* in a Sorvall T-1250 rotor. The final PM pellet was resuspended in a small volume of 10 mmol/L Tris buffer (200 μL/g of original tissue), frozen in liquid nitrogen, and stored at −80°C until analyzed. To assess the purity of the PM fractions, activity of the PM marker enzyme 5′-nucleotidase was measured[17] and compared with activity in the CH fraction. Aliquots of the PM (70 μg of protein) were treated with Laemmli sample buffer and subjected to SDS-PAGE run under reducing conditions on a 10% resolving gel. Resolved proteins were transferred to PVDF by the method of Towbin et al[15] using a Bio-Rad semidry transfer unit. GLUT4 and GLUT1 protein content of the plasma membrane was determined by Western blotting as described above.

IMTG

A portion of the quadriceps was homogenized 1:20 in 25 mmol/L KF/20 mmol/L EDTA buffer, pH 7.0, and subjected to lipid extraction as described by Burton et al.[18] Total triglyceride content of the lipid extract was determined using a commercially available kit (Infinity Triglycerides, Sigma-Aldrich, St Louis, MO).

Statistics

A 1-way analysis of variance was used on all variables to determine whether significant differences existed between 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, Muscle, and Epididymal Fat Pad Mass

At day 0 of the experimental treatment period, body mass of the CON, HF-LEP, and HF groups was similar (Table 1). Following the 12-day treatment period, the change in body mass of the HF-LEP group was significantly different from the CON and HF groups.

Table 1. Body, Muscle, and Epididymal Fat Pad Mass

	CON n = 8	HF-LEP n = 8	HF n = 8
Body mass (g)			
Treatment day 0	601.7 ± 34.6	657.7 ± 36.7	624.2 ± 30.0
Treatment day 12	618.9 ± 34.2	652.6 ± 36.1	640.7 ± 24.6
Δ Body weight	+ 17.2 ± 2.3	−4.8*† ± 4.9	+ 16.5 ± 6.7
Muscle mass (mg)			
Soleus	245.3 ± 10.3	240.9 ± 601	238.7 ± 10.5
Plantaris	547.1 ± 14.6	539.2 ± 17.7	548.0 ± 26.2
Epididymal fat pad mass (g)	12.2 ± 1.3	18.3* ± 1.7	21.2* ± 2.2

NOTE. Values are means ± SE.

Abbreviations: CON, control; HF-LEP, high-fat diet-leptin-treated; HF, high-fat diet.

*Significantly different from CON ($P < .05$); †significantly different from HF ($P < .05$).

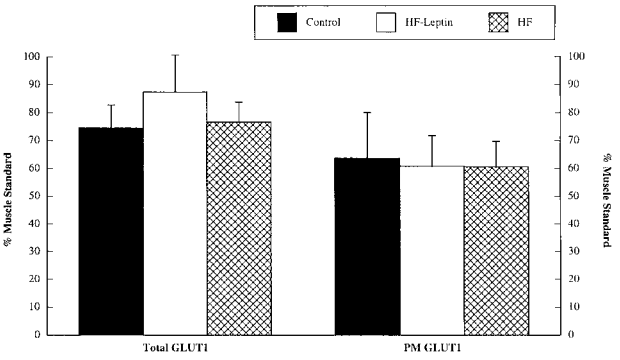

Fig 2. Total skeletal muscle (100 μg protein) and plasma membrane (PM, 100 μg protein) GLUT1 protein concentration, expressed as a percentage of muscle standard. Rats were divided among 1 of 3 groups: CON, HF-LEP, and HF. Values are means ± SE.

The mass of the Sol and Plant was similar across the 3 treatment groups (Table 1). Epididymal fat pad mass was similar between the HF-LEP and HF groups (Table 1). CON animals had significantly lighter epididymal fat pads compared with both the HF-LEP and HF animals.

3-MG Transport

Rates of non–insulin-stimulated 3-MG transport in the Sol and Plant of the HF animals was significantly lower than the CON animals (Fig 1). In contrast, 3-MG transport in the Sol and Plant of the HF-LEP animals was not different from the CON animals. No differences in basal 3-MG transport existed in the WG and RG among the CON, HF-LEP, and HF animals, although there was a trend ($P = .08$) for 3-MG transport in the RG to be lower in the HF versus CON animals.

Skeletal Muscle GLUT1 and GLUT4 Protein Concentration

Total skeletal muscle and plasma membrane GLUT1 protein concentrations were not different in the quadriceps of the CON, HF-LEP, and HF animals (Fig 2). Total skeletal muscle and plasma membrane GLUT4 protein concentrations in the quadriceps from the CON and HF-LEP animals were significantly greater compared with the HF group (Fig 3). No differences in total and plasma membrane GLUT4 protein concentrations existed in the quadriceps from the CON and HF-LEP animals. Assessment of 5′-nucleotidase activity (μmol/min/mg protein) indicated that the PM fractions were purified compared with the CH (CON, 50.2 ± 3.8 *v* 143.7 ± 10.0; HF-LEP, 40.0 ± 5.8 *v* 152.1 ± 12.6; HF, 33.2 ± 3.1 *v* 169.6 ± 9.3).

IMTG Content

A 3-month high-fat diet significantly increased IMTG content as evidenced in the HF group compared with CON animals (Fig 4). Of interest, a 12-day leptin treatment period significantly reduced IMTG levels such that the IMTG of the CON and HF-LEP animals were similar.

DISCUSSION

It is well established that when rodents consume a high-fat diet that they will exhibit skeletal muscle insulin resistance.[10,19-21] However, it has not been extensively investigated

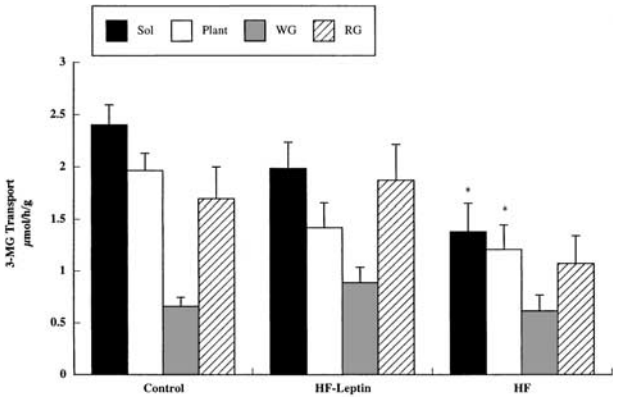

Fig 1. 3-MG transport in hind limb muscles of control (CON) (n = 8), high-fat diet-leptin-treated (HF-LEP) (n = 8), and high-fat diet (HF) (n = 8) rats. Sol, soleus; Plant, plantaris; WG, white gastrocnemius; RG, red gastrocnemius. Values are means ± SE. *Significantly different from CON ($P < .05$).

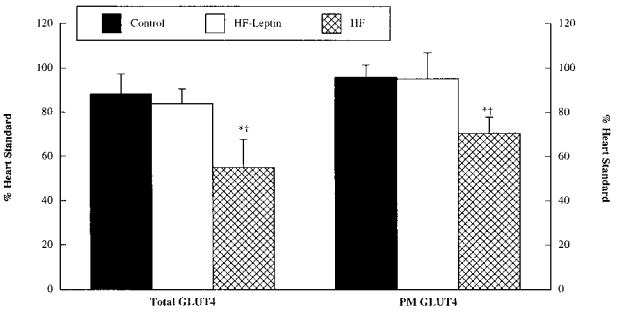

Fig 3. Total skeletal muscle (70 μg protein) and PM (100 μg protein) GLUT4 protein concentration, expressed as a percentage of a heart standard. Values are means ± SE. *Significantly different from CON ($P < .05$). †Significantly different from HF-LEP ($P < .05$).

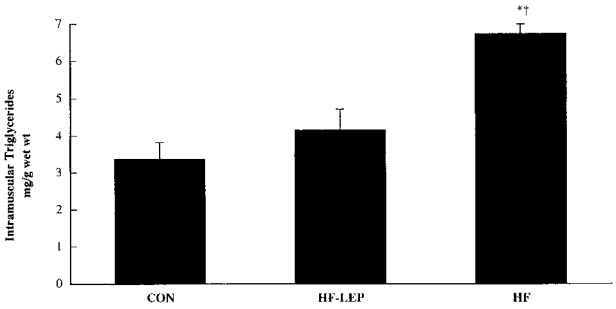

Fig 4. IMTG concentration in quadriceps from CON, HF-LEP, and HF animals. Values are means ± SE. *Significantly different from CON ($P < .05$). †Significantly different from HF-LEP ($P < .05$).

if a high-fat diet will also impair basal glucose transport in skeletal muscle. In one of the few investigations that have addressed this question, Hansen et al[19] reported that basal rates of 3-MG transport in epitrochlearis muscle, a predominately type IIb fiber muscle,[22] were not affected in rodents subjected to 8 weeks of a high-fat diet. In agreement with Hansen et al,[19] we observed that rates of basal 3-MG transport were not significantly different in the WG, a type IIb muscle fiber, of the HF animals when compared with the CON animals. However, we did observe that basal rates of 3-MG transport were significantly reduced in the Sol and Plant of the HF animals, which are predominately type I and type IIa fiber muscles,[23] respectively.

It was unclear why the basal rates of 3-MG transport were lower in these muscles of the HF animals, particularly since the total and plasma membrane protein concentration of the glucose transporter that regulates basal glucose transport (GLUT1) was not different among the groups. Halseth et al[24] recently evaluated the effects of a high-fat diet on basal and insulin–stimulated glucose uptake in rodent muscle and observed fiber type-specific effects. These investigators reported that a high-fat diet impairs glucose phosphorylation under basal conditions in the slow twitch soleus to a greater extent when compared with fast-twitch muscle fibers and contributes to reducing basal rates of glucose uptake. Of interest, we have previously observed that high–fat-fed rodents exhibit a reduced hexokinase activity in the soleus, but not in the plantaris, RG, or WG.[10] Thus, the differences observed in the muscles of the HF animals are likely due to the high-fat diet affecting basal carbohydrate metabolism in a fiber-type-specific manner.

We next evaluated if chronic leptin administration altered rates of basal 3-MG transport in the muscle of the high–fat-fed rodents. While chronic leptin administration has been shown to improve skeletal muscle insulin resistance,[10,25] it has not been reported if this intervention affects basal skeletal muscle glucose metabolism in muscle from high-fat–fed rats. Based on the present investigation, it appears that 12 days of leptin administration can normalize basal rates of 3-MG transport in specific muscles (ie, Sol and Plant) from high-fat–fed rats. While the Sol and Plant appeared to be more susceptible to the effects of the high-fat diet and the subsequent leptin treatment than did the WG and RG, this response is not atypical. We have previously shown leptin to have a greater effect on oxidative as opposed to glycolytic muscle fiber types.[10]

As we have previously used this rodent model to induce skeletal muscle insulin resistance,[10] we did not assess insulin-stimulated skeletal muscle glucose metabolism in the present investigation. However, to confirm the effects of the high-fat diet, we quantified the total and plasma membrane GLUT4 protein concentration. We observed that the high-fat diet reduced the total skeletal muscle GLUT4 protein concentration and is consistent with the findings of previous investigations.[19,26] We also observed that the GLUT4 protein concentration in the plasma membrane was reduced in the HF animals under basal conditions. Previous investigations have shown insulin-stimulated skeletal muscle 3-MG glucose transport rates to be related to the total GLUT4 protein concentration.[22,27-29] Therefore, it is plausible to suggest that since the skeletal muscle GLUT4 protein concentration was reduced by the high-fat diet in the HF animals that the skeletal muscle of these animals was also insulin-resistant.[19,24,30]

Why chronic leptin administration normalized basal 3-MG transport rates and GLUT4 protein concentration in the HF-LEP animals is unknown, but may be related to the IMTG concentration being altered, which will shift muscle metabolism from lipid storage to fat oxidation,[25,31] and improve whole body glucose tolerance.[32] Leptin may also mediate its effect by attenuating the circulating concentration of tumor necrosis factor-α (TNF-α) level.[33] TNF-α is secreted from highly active adipocytes in the abdominal region, and serum TNF-α levels have been reported to be elevated in obese, type 2 diabetics.[34] An excess of TNF-α attenuates in vitro expression of GLUT4[35] and impairs insulin-stimulated glucose uptake in C_2C_{12} muscle cells.[36]

An alternative possibility is that leptin is exerting a primary effect in skeletal muscle. Skeletal muscle expresses the long and short isoform of the leptin receptor,[5-7] and both isoforms have signal transduction capabilities.[6,37] The leptin receptor is a member of the gp130 family of cytokine receptors, which stimulate gene transcription via activation of cytosolic STAT proteins.[38] While it has not been reported if leptin activates these pathways in skeletal muscle, it is possible that leptin treatment may initiate an increased expression of skeletal muscle GLUT4 protein.

In summary, we observed that a high-fat diet impaired and chronic leptin administration normalized non–insulin-stimulated skeletal muscle glucose transport, but in a fiber type-specific manner. However, the changes in basal glucose transport could not be accounted for by alterations in the skeletal muscle GLUT1 protein concentration. In contrast a high-fat diet reduced and subsequent leptin treatment normalized the total skeletal muscle and plasma membrane GLUT4 protein concentration. These findings demonstrate a high-fat diet and chronic leptin administration alter basal carbohydrate metabolism in rodent skeletal muscle and differentially affect the GLUT1 and GLUT4 transporter concentration.

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