High-Fat Diet and Leptin Treatment Alter Skeletal Muscle Insulin-Stimulated Phosphatidylinositol 3-Kinase Activity and Glucose Transport

Mohenish K. Singh, Adam D. Krisan, Andrew M. Crain, Dale E. Collins, and Ben B. Yaspelkis III

The aim of this investigation was to evaluate if leptin treatment enhances insulin-stimulated glucose transport in normal (experimental group [EXP]-1) and insulin-resistant skeletal muscle (EXP-2) by altering components of the insulin-signaling cascade and/or glucose transport pathway. In EXP-1, Sprague Dawley rats were assigned to control-chow fed (CON-CF) or leptin treated-chow fed (LEP-CF) groups. Animals were implanted with miniosmotic pumps, which delivered 0.5 mg leptin/kg/d to the LEP-CF animals and vehicle to CON-CF animals for 14 days. For EXP-2, Sprague-Dawley rats consumed normal (CON) or high-fat diets for 3 months. After the dietary lead in, the high-fat diet group was further subdivided into high-fat (HF) and high-fat, leptin-treated (HF-LEP) animals. HF-LEP animals were injected with leptin (0.5 mg leptin/kg/d) for 12 days, while the CON and HF animals were injected with vehicle. After the treatment periods, all animals were prepared for and subjected to hind limb perfusion. In EXP-1, leptin treatment increased insulin-stimulated skeletal muscle glucose transporter (GLUT4) translocation, which appeared to be due to increased phosphatidylinositol 3-kinase (PI3-K) activation and Akt phosphorylation. In EXP-2, the high-fat diet reduced insulin-stimulated glucose transport, in part, by impairing insulin-stimulated PI3-K activation and glucose transporter translocation. Leptin treatment reversed high-fat-diet-induced insulin resistance in skeletal muscle by restoring insulin receptor substrate (IRS)-1-associated PI3-K activity, total GLUT4 protein concentration, and glucose transporter translocation. Collectively, these findings suggest that leptin treatment will enhance components of both the insulin-signaling cascade and glucose transport effector system in normal and insulin-resistant skeletal muscle.

LEPTIN, A PRODUCT of the *ob* gene, has been shown to reduce fat mass, food intake, and improve carbohydrate metabolism.[1-3] These improvements were previously attributed to leptin suppressing the release of neuropeptide Y in the central nervous system. However, leptin receptor isoforms exist in tissues other than the hypothalamus,[4,5] and insulin action is improved in these tissues after leptin treatment.[1,6] Barzilai et al[1] found that 8 days of leptin administration to Sprague-Dawley rats improved insulin action by reducing hepatic glycogenolysis and by increasing whole body glucose uptake when compared with control animals. In an extension to these findings, we have shown that 14 days of leptin treatment substantially increases insulin-stimulated skeletal muscle glucose uptake and 3-O-methyl-D-glucose (3-MG) transport in normal[7] and insulin-resistant[8] rodent skeletal muscle. Of interest, it appeared that leptin treatment improved high-fat diet-induced skeletal muscle insulin resistance, in part, by normalizing the skeletal muscle glucose transporter (GLUT4) protein concentration.[8]

However, skeletal muscle insulin resistance is not always associated with deficiencies in GLUT4 protein concentration. Humans with non–insulin-dependent diabetes mellitus[9] and some animal models of genetic obesity[10-12] possess a normal skeletal muscle GLUT4 protein concentration, but exhibit reduced insulin-stimulated translocation of the glucose transporters to the plasma membrane. Therefore, these findings suggest that skeletal muscle insulin resistance also results from impairments in the insulin-signaling cascade.

While we have found that chronic leptin administration improves insulin-stimulated glucose transport in insulin-resistant skeletal muscle and is related to the normalization of the GLUT4 protein concentration,[8] it is possible that the observed improvements may partially be attributed to alterations in the insulin-signaling cascade as well. Leptin administration has been shown to improve IRS-1–associated phosphatidylinositol 3-kinase (PI3-K) activation in liver,[13] IRS-2–associated PI3-K activation in C_2C_{12} muscle cells,[14] and enhance insulin-stimulated association of the p85 subunit of PI3-K with IRS-1 in Fao cells.[3] Therefore, the primary aim of this investigation was to determine if chronic leptin administration improves skeletal muscle insulin resistance not only by enhancing the glucose transport pathway, but also by altering components of the insulin signaling cascade. However, to fully elucidate this question we deemed that it was necessary to initially characterize the effects of chronic leptin treatment on the insulin-signaling cascade and glucose transport pathway in normal rodent skeletal muscle. We then used our high-fat–fed rodent model[8,15] in conjunction with leptin treatment, to (1) assess the effects of a high-fat diet on components of the insulin-signaling cascade and glucose transport effector system and (2) determine if improvements in skeletal muscle insulin resistance induced by chronic leptin treatment result from a reversal of high-fat diet-induced alterations in the insulin-signaling cascade and/or the glucose transport system.

MATERIALS AND METHODS

Animals

Experimental group 1. Sixteen female Sprague-Dawley rats weighing ~240 to 250 g were obtained from Animal Technologies Limited (ATL, Fremont, CA) and randomly assigned to 1 of 2 groups: Control-Chow Fed (CON-CF; n = 8) or Leptin-Chow Fed (LEP-CF; n = 8). On arrival, the rats were housed 3 per cage in a temperature-controlled animal room (21°C) maintained on a 12:12-hour light-dark cycle. The rats were provided standard rat chow (BeeKay Feed; ATL)

From the Department of Kinesiology, California State University Northridge, Northridge, CA.

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Address reprint requests to Ben B. Yaspelkis, III, PhD, Department of Kinesiology, California State University Northridge, 18111 Nordhoff St, Northridge, CA 91330-8287.

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and water ad libitum. Two weeks before hind limb perfusions, all animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (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 had reservoir volumes of 2 mL and a nominal pumping rate of 5 μL/h that can be maintained for 14 days. Control animals were implanted with miniosmotic pumps filled with phosphate-buffered saline (PBS), while leptin animals were implanted with osmotic pumps filled with 1 mg/mL recombinant murine leptin (Amgen, Thousand Oaks, CA). After insertion of the osmotic pumps, the animals were housed individually.

Experimental group 2. Twenty-four male Sprague-Dawley rats approximately 6 weeks of age were obtained from ATL and were randomly assigned to 1 of 2 groups: Control (CON, n = 8) and High-fat (HF, n = 16) groups. Control animals received a diet composed of normal fat content (17% fat-derived calories, Cat #112386; Dyets, Bethlehem, PA) and HF animals ate a high fat content diet (59% fat-derived calories, Cat #112387; Dyets). Both the control and high-fat diet were provided in powder form. Rats were housed 2 per cage in a temperature-controlled animal room (21°C) maintained on a 12-hour light-dark cycle. Animals were provided the respective diets and water ad libitum for 12 weeks. After the 12-week period, the HF group was further subdivided into 2 groups: high-fat (HF, n = 8) and High-fat Leptin (HF-LEP, n = 8). The leptin group received leptin injections (5 mg/100g body weight) 2 times a day at 8 AM and 5 PM, while CON and HF animals received PBS injections for a 12-day experimental period. 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.[8]

Female rats were used in experimental group (EXP)-1, as we wanted to be able to compare our present investigation with our previous study in which we used female rats implanted with miniosmotic pumps to assess the effects of leptin in non–insulin-resistant skeletal muscle.[7] However, it has been demonstrated that male rodents are more susceptible to various modes of diet-induced insulin resistance when compared with female rats.[16,17] Therefore, we chose to use male rodents subjected to a high-fat diet in the second experimental group to optimize the development of skeletal muscle insulin resistance. But, due to the elevated body mass of the animals in the second experimental group, providing leptin via osmotic pumps would not have delivered an adequate daily dose of leptin and consequently necessitated delivery by twice daily injections, as was performed in our previous investigations.[8,15]

We have previously evaluated the effects of daily food consumption and leptin treatment in normal chow-fed[7] and high-fat–fed[8] animals to separate the effects of food restriction from leptin treatment. We have found that food restriction does not increase hind limb glucose uptake and 3-MG transport in normal skeletal muscle[7] or reverse high-fat diet-induced skeletal muscle insulin resistance.[8] Given that leptin-treated and pair-fed (food-restricted) animals consumed identical amounts of food in our previous investigations, differences in insulin-stimulated skeletal muscle glucose uptake and transport that existed between these 2 groups were attributed to the effects of chronic leptin treatment. Based on these observations, food-restricted animals were not deemed necessary for inclusion in the design of the present investigation.

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

Hind Limb Perfusions

After the experimental periods, all animals were prepared for hind limb perfusions. Animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (6.5 mg/100 g body weight). The plantaris (Plant), and portions of the red (RG) and white (WG) gastrocnemius were excised from the left leg and stored at −80°C until analysis. IRS-1, Akt, and total GLUT4 protein concentration were assessed in the RG and WG. Intramuscular triglyceride (IMTG) content was assessed in the Plant. Rats were perfused as previously detailed.[7,8] Perfusions were performed in the presence of 500 μU/mL of insulin concentration for all experimental groups. Over the first 20-minute period, the rate of glucose uptake was assessed in which the perfusate contained 8 mmol/L glucose. Subsequent to the determination of glucose uptake, the hind limb was washed out with glucose-free perfusate for 1 minute in preparation for the measurement of glucose transport. Glucose transport was assessed for an 8-minute period in which the perfusate contained an 8 mmol/L concentration of the nonmetabolizable glucose analog 3-O-methyl-D-glucose ([3-MG] 32 μCi 3-[^{3}H]MG/mmol, Perkin Elmer Life Sciences, Boston, MA) and 2 mmol/L mannitol (60 μCi D-[1-^{14}C]mannitol/mmol, PerkinElmer Life Sciences). Rates of glucose uptake and 3-MG transport were determined as previously described.[7,8] Immediately at the end of the transport period, the red and white portions of the gastrocnemius were excised from the right leg, blotted on gauze dampened in cold Krebs-Heinseleit buffer (KHB), and clamped frozen in tongs cooled in liquid N_2. The quadricep was excised and frozen whole. The muscles were stored at −80°C until analyzed. Muscles from the perfused leg were used to determine 3-MG transport rates, IRS-1–associated PI3-K activity, phosphorylated Akt (pAkt) content, and plasma membrane GLUT4 content.

IRS-1, Akt, and GLUT4 Protein Concentration

Muscles were weighed frozen and homogenized in an ice-cold lysis buffer (1:20 wt/vol) containing 135 mmol/L NaCl, 1 mmol/L $MgCl_2$, 2.7 mmol/L KCl, 20 mmol/L Tris (pH 8.0), 0.5 mmol/L sodium vanadate, 10 mmol/L sodium fluoride, 0.2 mmol/L phenylmethylsulfonyl fluoride, 1% Triton-X, 10% glycerol, and 10 μg/mL leupeptin. Muscles were homogenized on ice using a glass Pyrex homogenizer, then placed in a Micromax RF microcentrifuge (IEC, Needham Heights, MA) cooled to 4°C and centrifuged at 12,000 rpm for 10 minutes. The supernatant was extracted and quantified for protein content by the Bradford method.[18]

To determine IRS-1, Akt, or GLUT4 content, 100-μL sample lysate was diluted 1:1 with Laemmli sample buffer. Fifty micrograms of sample protein for the determination of IRS-1 and Akt and 35 μg for GLUT4 were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) run under reducing conditions on a 7.5% resolving gel (IRS-1 and Akt) or 12.5% resolving gel (GLUT4) on a Mini-Protean II dual slab cell (BioRad, Richmond, CA). Resolved proteins were transferred to polyvinylidene difluoride (PVDF) sheets (BioRad) by the method of Towbin et al[19] using a BioRad semidry transfer unit. The PVDF membranes were blocked in 5% nonfat dry milk/TTBS, incubated with affinity purified polyclonal α IRS-1 (1:500; sc-559, Santa Cruz Biotechnology, Santa Cruz, CA), α Akt 1/2 (1:500; sc-8312, Santa Cruz Biotechnology) or α GLUT-4 (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 horseradish peroxidase (HRP)-labeled protein A (1:2,000; Amersham Life Science, Arlington Heights, IL). Antibody binding was visualized using enhanced chemiluminescence autoradiography in accordance with the manufacturer's instructions (Amersham Life Science). Bands were quantified by capturing images of the autoradiographs with an image scanner (ScanJet 4C; Hewlett Packard, Boise, ID) equipped with a transparency module and a Macintosh G4

computer (Cupertino, CA). 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/) for quantification. The density of the bands was calculated as percentage of a standard (50 μg protein of a muscle standard for IRS-1 and Akt, or 15 μg protein of heart standard for GLUT4) run on each gel.

IRS-1–Associated PI3-K Activity

Muscles were weighed frozen and homogenized in an ice-cold homogenization buffer (1:10 wt/vol) containing 50 mmol/L HEPES (pH 7.6), 150 mmol/L NaCl, 200 mmol/L sodium pyrophosphate, 20 mmol/L B-glycerophosphate, 20 mmol/L NaF, 2 mmol/L sodium vanadate, 20 mmol/L EDTA (pH 8.0), 1% IGEPAL, 10% glycerol, 2 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L $MgCl_2$, 1 mmol/L CaCl2, 10 μg/mL leupeptin, and 10 μg/mL aprotinin. Muscles were homogenized using a glass Pyrex homogenizer, then centrifuged at 12,000 rpm at 4°C for 10 minutes. The supernatant was extracted and quantified for protein content by the Bradford method.[18]

Two-hundred fifty micrograms of sample protein was immunoprecipitated with 16 ng/μL α IRS-1 (Cat# 06-248; Upstate Biotechnology, Lake Placid, NY) and homogenization buffer for 2 hours on ice. Protein A-Sepharose Type CL-4B beads (Amersham Pharmacia Biotec, Piscataway, NJ) were prepared by washing once with 0.5% trichloroacetic acid and twice with homogenization buffer. Eighty microliters of Protein A-Sepharose was added to the αIRS-1 immunoprecipitates for 1.5 hours at 4°C with rotation. Samples were then centrifuged at 14,000 rpm for 10 minutes at 4°C in a Micromax RF microcentrifuge, and the immunocomplex was washed successively with the following: Buffer A [10% IGEPAL, 100 mmol/L sodium vanadate, 1 mol/L dithiothreitol (DTT), PBS], Buffer B [1 mol/L Tris-HCl (pH 7.5), 2 mol/L $LiCl_2$, 100 mmol/L sodium vanadate, 1 mol/L DTT], and Buffer C [1 mol/L Tris-HCl (pH 7.5), 5 mol/L NaCl, 100 mmol/L sodium vanadate, 10 mmol/L EDTA, 1 mol/L DTT]. Washing was performed once in buffer A and buffer B and twice in buffer C. Packed beads were suspended and incubated for 5 minutes in 20 μL phosphatidylinositol (Avanti Polar Lipids, Alabaster, AL). The kinase reaction was started by the addition of 10 μL 0.4 mol/L $MgCl_2$, 200 mmol/L HEPES (pH 7.6), 4 mmol/L EGTA, 4 mmol/L NaH_2PO_4, and [γ-^{32}P]adenosine triphosphate (ATP) (PerkinElmer Life Sciences). After 5 minutes of incubation at room temperature with vigorous shaking, the reaction was terminated by the addition of 15 μL 4 N HCl and 130 μL MeOH-$CHCl_3$ (1:1 vol/vol). After brief centrifugation, 40 μL of the organic solvent layer was spotted onto a thin-layer chromatography plate (Silica gel 60; Whatman, Hillsboro, OR). After separation of phosphoinositides in running solvent ($CHCl_3$-MeOH-H_2O-NH_4OH; 60:47:11:3.2), plates were dried and exposed to Kodak Biomax MR film (Rochester, NY). Spots were scraped from the plates, and radioactivity was counted using a liquid scintillation counter. Kinase activity was expressed as a percentage of an insulin-stimulated muscle standard run on each plate.

Phosphorylated Akt Concentration

Aliquots of muscle samples that were used for IRS-1 associated PI3-K activity were used to determine phosphorylated Akt (pAkt) content. One hundred micrograms of sample protein was subjected to SDS-PAGE run under reducing conditions on a 7.5% resolving gel. The resolved proteins were then transferred to PVDF membrane as previously described and blocked in 5% nonfat dry milk/TTBS. The membranes were incubated with an affinity purified polyclonal antibody specific for Ser 473 phosphorylated Akt 1(1:500; sc-7985, Santa Cruz Biotechnology) followed by incubation with HRP-labeled Protein A (1:10,000; Amersham Life Sciences). Antibody binding was visualized using chemiluminescence in accordance with the manufacturer's instructions (West Femto, Pierce Chemical, Rockford, IL). Images were captured with a ChemiDoc supercooled CCD camera system (BioRad) and saved to a Macintosh G4 computer. Bands were quantified using Quantity One analysis software (BioRad). The density of the bands was calculated as a percentage of a standard (100-μg protein of an insulin-stimulated muscle sample) run on each gel.

Plasma Membrane GLUT4 Protein Concentration

Plasma membrane fractions were prepared from portions of perfused mixed red and white quadriceps muscle according to the procedure of Turcotte et al.[20] 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, 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 and retained for analysis. The remaining homogenate suspension was layered on continuous sucrose gradients (35% to 70%) and centrifuged at 120,000 $\times$ *g* for 2 hours in a Sorvall Surespin 630/36 rotor. The plasma membrane 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 plasma membrane 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 plasma membrane fractions, protein content of the plasma membrane and activity of the plasma membrane marker enzyme 5′-nucleotidase was measured and compared with activity in the crude homogenate fraction. Aliquots of the plasma membrane (35 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 membranes as described previously and incubated with affinity-labeled polyclonal αGLUT-4 (1:1,000), followed by incubation with HRP-labeled protein A (1:10,000). The bands were visualized and captured as described for pAkt. The density of the bands was expressed as a percentage of a heart standard (15 μg of heart protein) run on each gel.

IMTG Content

IMTG content was assessed by homogenizing the mixed fiber type plantaris muscle (1:20 wt/vol) in buffer containing 50 mmol/L KF/1 mmol/L EDTA (pH 7.4), and subjected to lipid extraction as described by Burton et al.[21] 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 Mass

EXP-1. At day 0 of the experimental treatment, the body mass of the CON-CF (244.5 $\pm$ 4.5 g) and LEP-CF (250.2 $\pm$ 2.8 g) groups were similar. After the treatment period, the body mass of the LEP-CF animals (228.6 $\pm$ 3.9 g) was significantly

Table 1. Insulin-Stimulated Rates of Skeletal Muscle Glucose Uptake and 3-MG Transport

	Experimental Group 1		Experimental Group 2		
	CON-CF	LEP-CF	CON	HF	HF-LEP
Glucose uptake (μmol/g/h)	17.98 ± 1.3	24.82* ± 1.0	12.51 ± 0.6	6.23†‡ ± 0.7	12.06 ± 1.1
3-MG transport (μmol/h/g)					
RG	12.09 ± 1.2	15.18* ± 0.8	7.29 ± 0.7	4.83†‡ ± 1.2	8.11 ± 0.4
WG	3.79 ± 0.4	5.50 ± 0.6	0.93 ± 0.2	0.84 ± 0.1	1.68 ± 0.6

NOTE. Values are means ± SE.

Abbreviations: CON-CF, control-chow fed; LEP-CF, leptin-treated chow fed; CON, control; HF, high-fat diet; HF-LEP, high-fat diet leptin treated; RG, red gastrocnemius; WG, white gastrocnemius.

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

lower ($P < .05$) when compared with CON-CF animals (271.2 ± 6.3 g).

EXP-2. At the beginning of the experimental treatment period, the body mass of the CON group (607.5 ± 24.4 g) was significantly lighter than both the HF-LEP (753.9 ± 32.7 g) and HF groups (746.9 ± 27.9 g). After the leptin treatment period, the body mass of the HF-LEP animals was reduced ~12g. However, the body mass of the HF-LEP (741.6 ± 33.9 g) and HF (746.9 ± 29.0 g) animals remained greater than that of the CON animals (606.5 ± 23.7 g) at the end of the treatment period.

Glucose Uptake and 3-MG Transport

EXP-1. Leptin administration elevated rates of hind limb glucose uptake in the LEP-CF group when compared with the CON group (Table 1). 3-MG transport rates were found to be significantly elevated in the RG of the LEP-CF animals compared with CON-CF (Table 1), but no differences in 3-MG transport rates were found in the WG between the groups.

EXP-2. A high-fat diet significantly decreased the rate of insulin-stimulated hind limb glucose uptake, as was evidence when the HF and CON groups were compared (Table 1). In contrast, no differences existed in rates of insulin-stimulated glucose uptake between the CON and HF-LEP groups. Rates of 3-MG transport in the RG were significantly reduced in the HF group compared with both the CON and HF-LEP groups (Table 1). However, there were no differences in the rates of 3-MG transport in the RG of the CON and HF-LEP animals. Rates of 3-MG transport in the WG of the CON, HF-LEP, and HF groups were similar.

Insulin-Signaling Protein Concentration and Activation

EXP-1. Total IRS-1 protein (Fig 1A) and Akt (Fig 2A) concentration were similar in the RG of the CON-CF and LEP-CF animals. However, IRS-1–associated PI3-K activity (Fig 3A) and phosphorylation of Akt (Fig 4A) were significantly increased in the RG of the leptin-treated animals. In the WG, no differences in IRS-1 protein concentration, Akt protein concentration, IRS-1–associated PI3-K activity, or Akt phosphorylation existed between the CON-CF and LEP-CF animals.

EXP-2. Although the high-fat diet did not affect total IRS-1 (Fig 1B) or Akt (Fig 2B) protein concentrations in the RG, it did significantly reduce IRS-1–associated PI3-K activity (Fig 3B). Leptin treatment increased the IRS-1 protein in the RG, while not altering Akt protein concentration. Of interest, leptin treatment normalized PI3-K activation in the RG as evidence that no differences existed between the CON and HF-LEP groups. Furthermore, we observed Akt phosphorylation (Fig 4B) to be elevated in the RG of the HF-LEP group compared with both the CON and HF animals. In the WG, no differences existed among groups for IRS-1 protein concentration, Akt

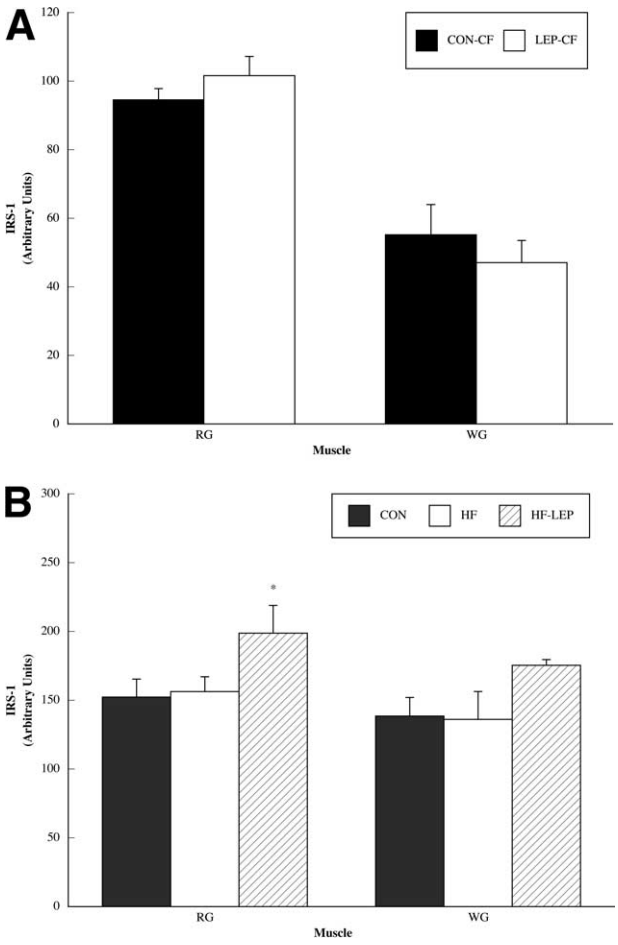

Fig 1. Skeletal muscle IRS-1 protein concentration. (A) EXP-1: control chow fed (CON-CF) and leptin-treated chow fed (LEP-CF). (B) EXP-2: control (CON), high-fat diet (HF), and high-fat diet leptin-treated (HF-LEP). *Significantly different from CON ($P < .05$). Values are means ± SE. RG, red gastrocnemius; WG, white gastrocnemius.

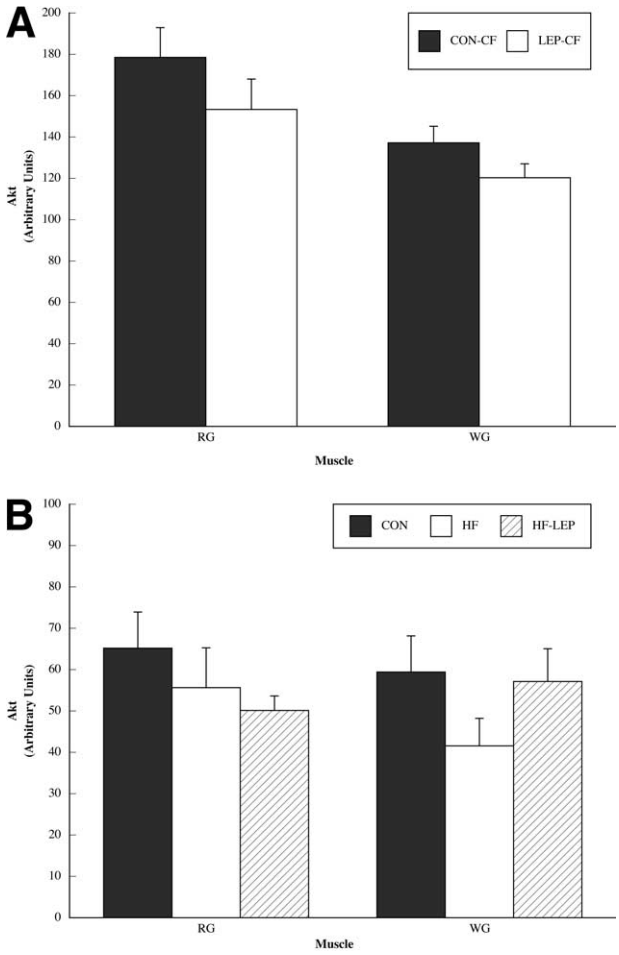

Fig 2. Skeletal muscle Akt protein concentration. (A) EXP-1: CON-CF and LEP-CF. (B) EXP-2: CON, HF, and HF-LEP treated. Values are means ± SE.

protein concentration, IRS-1–associated PI3-K activity, or Akt phosphorylation.

Glucose Transporters

EXP-1. Although no differences existed between groups for total GLUT4 protein concentration in either the RG and WG (Fig 5A), leptin treatment resulted in a significantly greater plasma membrane GLUT4 protein concentration in response to insulin stimulation (Fig 6A). Assessment of 5′-nucleotidase activity (μmol/min/mg protein) indicated that the plasma membrane fractions were purified compared with the crude homogenate (CON-CF: 35.4 ± 2.2 *v* 98.3 ± 4.6; LEP-CF: 34.5 ± 2.2 *v* 124.4 ± 10.2).

EXP-2. The high-fat diet significantly reduced the total GLUT4 protein concentration in the RG of the HF animals when compared with the CON animals (Fig 5B). Leptin treatment reversed the effects of the high-fat diet, as it was found that total GLUT4 protein concentration in the RG was similar between the CON and HF-LEP animals. No differences in total GLUT4 protein concentration were found in the WG among groups.

Insulin-stimulated plasma membrane GLUT4 protein concentration from the perfused quadricep was significantly lower in the HF animals compared with the CON animals (Fig 6B). In contrast, insulin-stimulated plasma membrane GLUT4 protein concentration was normalized in the HF-LEP animals (Fig 6B). Plasma membrane fractions were purified in comparison to the crude homogenates as determined by 5′-nucleotidase activity (μmol/min/mg protein) (CON: 24.7 ±1.4 *v* 146.4 ± 5.0; HF: 28.4 ± 2.1 *v* 130.2 ± 20.7; HF-LEP: 28.1 ± 2.0 *v* 153.8 ± 10.5).

IMTG Content

EXP-1. IMTG content in the LEP-CF animals (1.97 ± 0.14 mg/g wet weight) was significantly reduced ($P < .05$) compared with CON-CF animals (2.28 ± 0.17 mg/g wet weight).

EXP-2. The high-fat diet significantly elevated ($P < .05$) IMTG as evident in the HF group (3.68 ± 0.53 mg/g wet weight) compared with CON animals (2.52 ± 0.14 mg/g wet weight). Leptin treatment significantly reduced ($P < .05$)

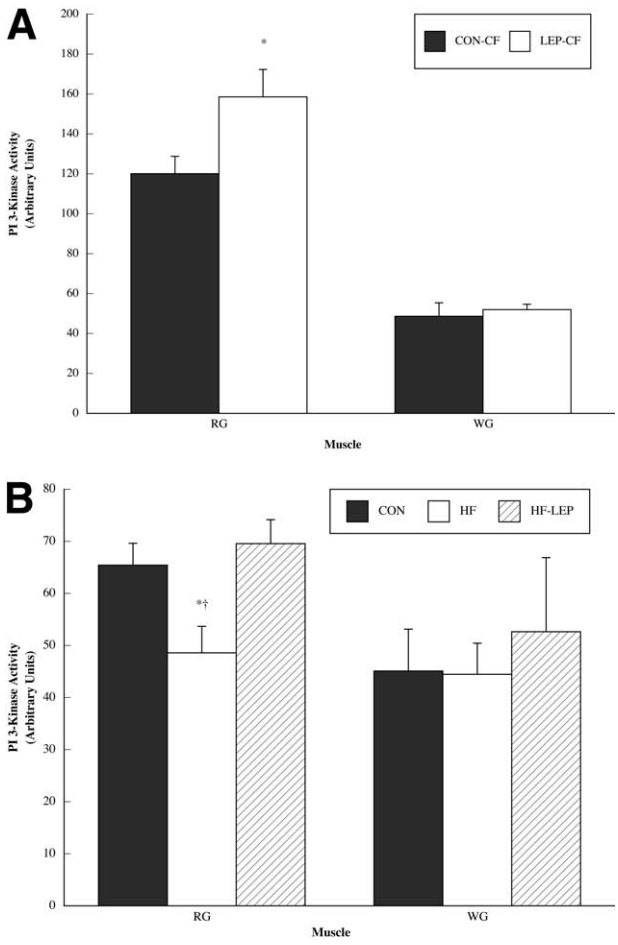

Fig 3. Skeletal muscle insulin-stimulated IRS-1–associated PI3-K activity. (A) EXP-1: CON-CF and LEP-CF. *Significantly different from CON-CF ($P < .05$). (B) EXP-2: CON, HF, and HF-LEP treated. *Significantly different from CON ($P < .05$); †significantly different from HF-LEP ($P < .05$). Values are means ± SE.

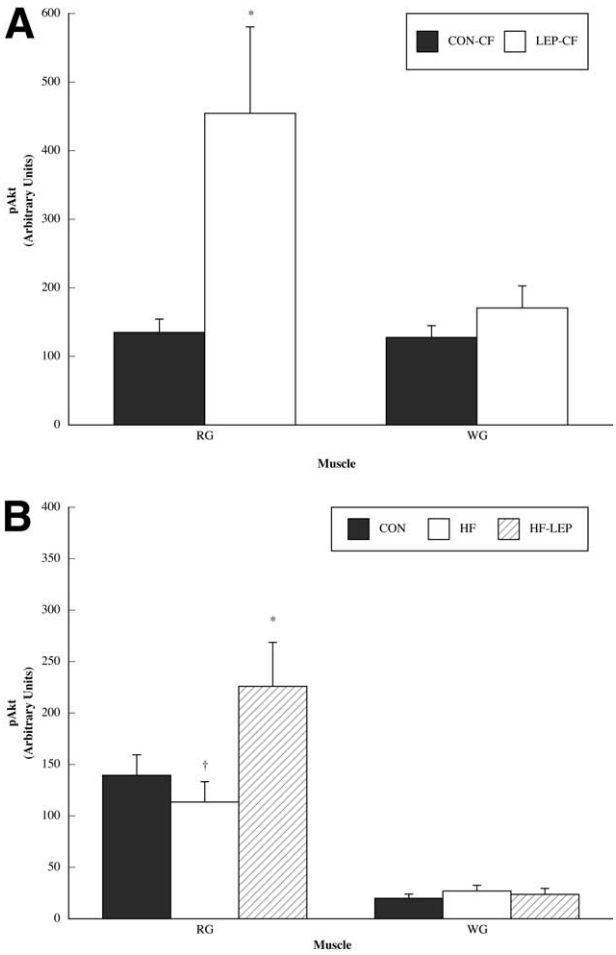

Fig 4. Phosphorylated Akt concentration in insulin-stimulated skeletal muscle. (A) EXP-1: CON-CF and LEP-CF. *Significantly different from CON-CF ($P < .05$). (B) EXP-2: CON, HF, and HF-LEP treated. *Significantly different from CON ($P < .05$); †significantly different from HF-LEP ($P < .05$). Values are means ± SE.

IMTG levels such that the IMTG of CON and HF-LEP (2.53 ± 0.25 mg/g wet weight) animals were similar.

DISCUSSION

Although our primary intent was to evaluate the effects of leptin treatment on insulin-resistant skeletal muscle, we deemed that it was necessary to initially characterize the effects of chronic leptin treatment on non–insulin-resistant skeletal muscle such that we could evaluate the effects of leptin on the insulin-signaling cascade and the glucose transport pathway in the absence of the confounding effects of a high-fat diet. To assess the effect of leptin treatment on skeletal muscle carbohydrate metabolism, we used the hind limb perfusion technique and found that leptin treatment resulted in significant improvements in insulin-stimulated glucose uptake in normal skeletal muscle, which is consistent with what we[7] and others[22] have previously reported. Furthermore, we found that leptin treatment enhanced rates of 3-MG transport in the RG (a type IIa fiber), but not in the WG (a type IIb fiber), suggesting that leptin does not affect the glucose transport pathway in glycolytic muscle fibers, which we have previously reported.[7,8]

We[7] had previously attributed leptin-induced improvements on insulin-stimulated 3-MG transport in normal skeletal muscle to an increased GLUT4 protein concentration, as it has been reported that there is a direct relationship between the total GLUT4 protein concentration and rates of 3-MG transport.[10,11,23] Subsequently, we found that chronic leptin treatment normalizes the total GLUT4 protein concentration in insulin-resistant skeletal muscle.[8] Therefore, we initially chose to evaluate the total skeletal muscle GLUT4 protein concentration to ascertain if improvements in 3-MG transport might be related to alterations in the GLUT4 protein concentration. Surprisingly, the increases in 3-MG transport in the RG could not be accounted for by alterations in total GLUT4 protein concentration, as we observed that the total GLUT4 protein concentration in the RG was similar between the LEP-CF and CON-CF animals (Fig 6A). Consequently, while the number of glucose transporters that are translocated to the plasma membrane in response to insulin have been observed to be related to the total pool of glucose transporters,[24,25] it appears that this

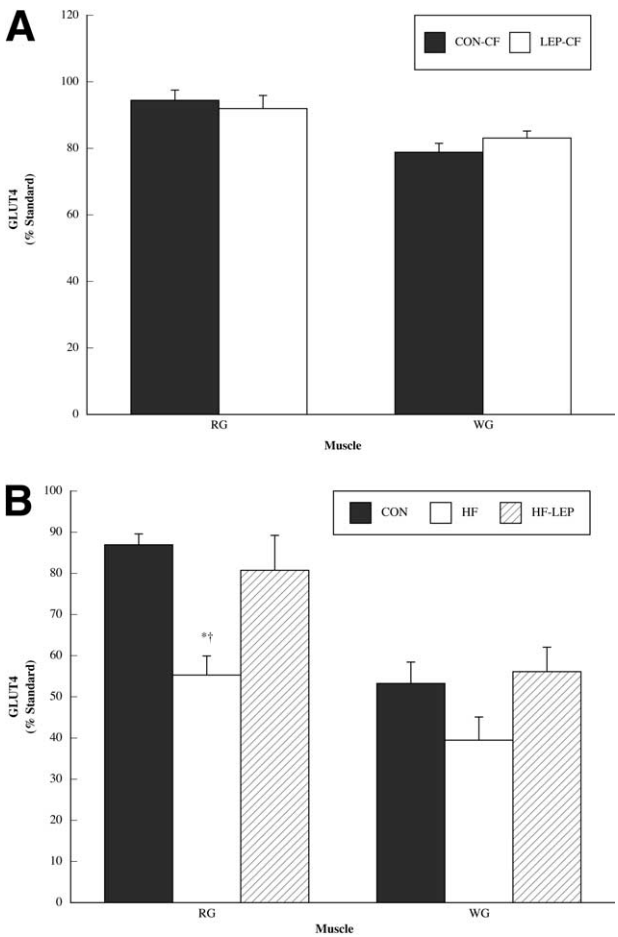

Fig 5. Total skeletal muscle GLUT4 protein concentration. (A) EXP-1: CON-CF and LEP-CF. (B) Experimental group 2: CON, HF, and HF-LEP treated. *Significantly different from CON ($P < .05$); †significantly different from HF-LEP ($P < .05$). Values are means ± SE.

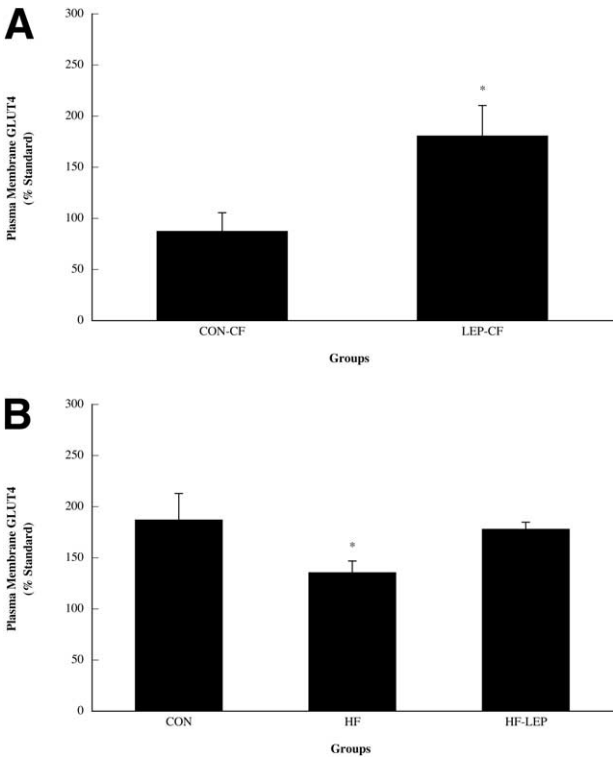

Fig 6. Insulin-stimulated plasma membrane GLUT4 protein concentration. (A) EXP-1: CON-CF and LEP-CF. *Significantly different from CON-CF ($P < .05$). (B) EXP-2: CON, HF, and HF-LEP treated. *Significantly different from CON ($P < .05$). Values are means ± SE.

relationship can be disassociated. To address this possibility, we assessed the plasma membrane GLUT4 protein concentration in the quadricep of the perfused hind limb and found that chronic leptin treatment enhanced insulin-stimulated GLUT4 protein translocation to the plasma membrane, which may partially account for the increased rates of 3-MG transport in the RG of the LEP-CF animals. Based on this observation, it was clear that our next line of inquiry should be directed towards determining how chronic leptin treatment increased insulin-stimulated GLUT4 translocation to the plasma membrane in normal skeletal muscle.

Increases in insulin-stimulated glucose uptake, 3-MG transport, and GLUT4 protein translocation have been attributed to improvements in insulin signal transduction.[6,26,27] In a recent review, Ceddia et al[28] indicated the potential for "cross talk" to occur between leptin and the insulin signaling pathways. It should be noted, however, that those studies, which have evaluated this possibility, have used cell lines treated acutely with leptin. To the best of our knowledge, the present investigation is the first to assess the effects of chronic leptin administration on components of the insulin-signaling cascade in rodent skeletal muscle. However, due to the complexity of the insulin-signaling cascade, we chose to initially limit our evaluation of potential alterations in this signaling cascade to several proteins associated with PI3-K, as it has been shown that activation of PI3-K is absolutely required for glucose transporter translocation.[6,27,29] Chronic leptin treatment did not alter the expression of either IRS-1 or Akt protein in normal skeletal muscle. Despite no change in the expression of these proteins, we did find that IRS-1–associated PI3-K activity and the downstream activation of Akt were enhanced in leptin-treated skeletal muscle. It is not readily apparent why chronic leptin treatment increased IRS-1–associated PI3-K activity, but is not without precedence. While difficult to draw direct comparisons to the present investigation, Kellerer et al[30] have found that acute leptin treatment increases insulin-stimulated IRS-2–associated PI3-K activation in C_2C_{12} cells. In addition, Szanto et al[3] have reported that when Fao cells are acutely treated with leptin that insulin enhances PI3-K activity due to an increased association of the regulatory p85 subunit of PI3-K with IRS-1 and, in turn, increases serine phosphorylation of Akt.

Having established the effect of chronic leptin treatment on normal skeletal muscle, our next line of inquiry was to evaluate if chronic leptin treatment alleviated high-fat diet-induced alterations in skeletal muscle insulin signaling and/or glucose transport. Again using the hind limb perfusion model to assess the effects of a high-fat diet on insulin-stimulated skeletal muscle carbohydrate, we found that both rates of hind limb glucose uptake and 3-MG transport in the RG were significantly reduced in the HF animals compared with the CON animals. This observation is in excellent agreement with a number of previous investigations that have also shown a high-fat diet to decrease insulin-stimulated glucose uptake and 3-MG transport in rodent skeletal muscle.[8,31-36] Reduced rates of insulin-stimulated glucose transport in the skeletal muscle of high-fat–fed rodents have been attributed to a reduced total GLUT4 protein concentration and/or an inability to effectively translocate glucose transporters to the plasma membrane.[8,32,37] Consistent with these observations, we found that both total and insulin-stimulated plasma membrane GLUT4 protein concentration were reduced in the skeletal muscle of the HF animals.

Although the high-fat diet decreased rates of insulin-stimulated 3-MG transport and total GLUT4 protein concentration in the oxidative muscle fibers, the high-fat diet did not affect IRS-1 (Fig 1B) or Akt protein concentrations (Fig 2B). Then again, this was not completely unexpected, as it has been reported in various models of insulin resistance that neither IRS-1 protein concentration[38,39] nor Akt protein concentration[37,39,40] are altered. However, the high-fat diet did decrease insulin-stimulated PI3-K activity (Fig 3B), which is consistent with previous investigations that have used high-fat feeding.[37,41] Moreover, reduced PI3-K activity appears to be universally characteristic in the skeletal muscle from a variety of insulin-resistant models.[37,39,42,43] While Akt has been implicated as a downstream target of the phosphoinositides generated by PI3-K, it is unclear how essential Akt activation is for glucose transporter translocation to occur. Akt activation has been reported to be reduced[37,39,40] or unchanged[42-45] in various models of insulin resistance. Recently, Nadler et al[42] showed that Akt activation was normal in insulin-resistant adipocytes obtained from BtB6 insulin-resistant mice despite PI3-K activation being substantially reduced. From this observation, these investigators concluded that Akt activation is not necessary to promote glucose transport. In agreement with these findings, we found that a high-fat diet did not alter insulin-stimulated Akt activation in skeletal muscle even though IRS-1–associated

PI3-K activity and glucose transporter translocation were reduced.

Having now established that our high-fat model induced skeletal muscle insulin resistance by altering both the insulin-signaling cascade and glucose transport pathway, we next evaluated how chronic leptin treatment reversed the effects of the high-fat diet. Although we[8] previously reported that chronic leptin administration normalizes insulin-stimulated glucose uptake and transport in high-fat–fed rodent skeletal muscle, which we observed again in the present investigation, we likely had not fully elucidated the mechanism of action. We had suggested that the leptin-induced improvements on rates of insulin-stimulated 3-MG transport were due, in part, to leptin normalizing the total skeletal muscle GLUT4 protein concentration. We again show that chronic leptin treatment normalized both the total (Fig 5B) and insulin-stimulated plasma membrane GLUT4 protein concentrations (Fig 6B) in the skeletal muscle of rodents fed a high-fat diet. However, in light of the effects of the high-fat diet impairing components of the insulin-signaling cascade, it was likely that the leptin-induced improvements on insulin-stimulated 3-MG transport in the high-fat–fed rodent skeletal muscle may have resulted from alterations in the insulin-signaling cascade as well. Consistent with this hypothesis, IRS-1 protein concentration (Fig 1B) and Akt activation (Fig 4B) were increased, while IRS-1–associated PI3-K activity (Fig 3B) was normalized in the in the skeletal muscle of the HF-LEP animals. However, as previously discussed, it is unlikely that enhanced Akt activation contributed substantially to the altered rates of 3-MG transport in the leptin-treated skeletal muscle.

While it is quite evident that chronic leptin treatment improved the high-fat diet-induced skeletal muscle insulin resistance through alterations in the components of the insulin-signaling cascade and glucose transport pathway, it is not immediately clear how leptin manifested these effects. While it is reasonable to suggest that chronic leptin administration may have improved insulin-stimulated glucose uptake and transport due to reductions in visceral fat concentration, the effect of leptin administration on peripheral insulin action cannot be explained solely by decreases in visceral fat deposition.[8,15,46] Therefore, the possibility exists that secondary effects in response to leptin treatment may have accounted for these effects. Leptin administration alters skeletal muscle metabolism by shifting the muscle from lipid storage to fat oxidation[47] and decreases fatty acid translocase (FAT) and fatty acid binding protein (FABP),[48] which could collectively decrease the IMTG content. A reduction in skeletal muscle triglyceride levels improves whole body glucose tolerance[49] and is consistent with our finding that leptin administration reduced IMTG levels.

Alternatively, chronic leptin treatment may have counteracted the effects of a high-fat diet due to leptin's ability to lessen the impact of elevated blood tumor necrosis factor-α (TNF-α) levels.[50] It has been reported that a high-fat diet given to C57BL/6J mice for 13 weeks will significantly increase adipocyte TNF-α secretion.[51] When TNF-α is in excess, it can attenuate in vitro expression of GLUT4,[52] insulin receptor tyrosine kinase activity,[53] insulin-stimulated glucose uptake in C_2C_{12} muscle cells,[54] and insulin signaling.[55,56] Insulin stimulation typically causes phosphorylation of IRS-1 tyrosine residues, but when IRS-1 serine and threonine residues are phosphorylated, insulin signaling is impaired.[55] Of interest, Paz et al[55] reported that when Fao cells were incubated with TNF-α that this cytokine phosphorylated the IRS-1 serine and threonine residues, which prevented the binding of IRS-1 to the juxtamembrane region of the insulin receptor and inhibited insulin action. Collectively, these data provide a plausible explanation that could account for the decreased insulin-stimulated activation of PI3-K in the HF animals and, conversely, the normalization of insulin-stimulated IRS-1–associated PI3-K activity in the HF-LEP animals.

In summary, leptin administration significantly increased insulin-stimulated glucose uptake and 3-MG transport in normal skeletal muscle due to IRS-1–associated PI3-K activity being enhanced, which in turn, increased the number of glucose transporters that were transported to the plasma membrane. A high-fat diet induced skeletal muscle insulin resistance by decreasing IRS-1–associated PI3-K activity and the total GLUT4 protein concentration leading to reduced glucose transporter translocation. Chronic leptin treatment reversed a high-fat diet-induced skeletal muscle insulin resistance by normalizing the total skeletal muscle GLUT4 protein concentration and PI3-K activation. Collectively, these alterations in the leptin-treated muscle resulted in the GLUT4 protein being more effectively translocated to the plasma membrane in response to insulin stimulation, thereby normalizing rates of glucose transport.

REFERENCES

1. Barzilai N, Wang J, Massilon D, et al: Leptin selectively decreases visceral adiposity and enhances insulin action. J Clin Invest 100:3105-3110, 1997

2. Pan DA, Lillioja S, Kriketos AD, et al: Skeletal muscle triglyceride levels are inversely related to insulin action. Diabetes 46:983-988, 1997

3. Szanto I, Kahn CR: Selective interaction between leptin and insulin signaling pathways in a hepatic cell line. Proc Natl Acad Sci USA 97:2355-2360, 2000

4. Berti L, Gammeltoft S: Leptin stimulates glucose uptake in C2C12 muscle cells by activation of ERK2. Mol Cell Endocrinol 157:121-130, 1999

5. Bjorbaek C, Uotani S, da Silva B, et al: Divergent signaling capacities of the long and short isoforms of the leptin receptor. J Biol Chem 272:32686-32695, 1997

6. Cheatham B, Vlahos CJ, Cheatham L, et al: Phosphatidylinositol 3-kinase activation is required for insulin stimulation of pp70 S6 kinase, DNA synthesis, and glucose transporter translocation. Mol Cell Biol 14:4902-4911, 1994

7. Yaspelkis BB III, Ansari L, Ramey EA, et al: Chronic leptin administration increases insulin-stimulated skeletal muscle glucose uptake and transport. Metabolism 48:671-676, 1999

8. Yaspelkis BB III, Davis JR, Saberi M, et al: Leptin administration improves skeletal muscle insulin responsiveness in diet-induced insulin-resistant rats. Am J Physiol 280:E130-E142, 2001

9. Lund S, Vestergaad H, Anderson PH, et al: GLUT-4 content in plasma membrane of muscle from patients with non-insulin-dependent diabetes mellitus. Am J Physiol 265:E889-E897, 1993

10. Banks EA, Brozinick JT Jr, Yaspelkis BB III, et al: Muscle

glucose transport, GLUT4 content and degree of exercise training in obese Zucker rats. Am J Physiol 263:E1010-E1015, 1992

11. Friedman JE, Sherman WM, Reed MJ, et al: Exercise training increases glucose transporter GLUT-4 in skeletal muscle of obese Zucker (fa/fa) rats. FEBS Lett 268:13-16, 1990

12. Koranyi L, James D, Mueckler M, et al: Glucose transporter levels in spontaneously obese (db/db) insulin-resistant mice. J Clin Invest 85:962-967, 1990

13. Anai M, Funaki M, Ogihara T, et al: Altered expression levels and impaired steps in the pathway to phosphatidylinositol 3-kinase activation via insulin receptor substrates 1 and 2 in Zucker fatty rats. Diabetes 47:13-23, 1998

14. Berti L, Kellerer M, Capp E, et al: Leptin stimulates glucose transport and glycogen synthesis in C_2C_{12} myotubes: Evidence for a PI 3-kinase mediated effect. Diabetologia 40:606-609, 1997

15. Yaspelkis BB III, Saberi M, Singh MK, et al: Chronic leptin treatment normalizes basal glucose transport in a fiber type-specific manner in high-fat–fed rats. Metabolism 51:859-863, 2002

16. Hevener A, Reichart D, Janez A, et al: Female rats do not exhibit free fatty acid-induced insulin resistance. Diabetes 51:1907-1912, 2002

17. Kim J, Nolte LA, Hansen PA, et al: Insulin resistance of muscle glucose transport in male and female rats fed a high-sucrose diet. Am J Physiol 276:R665-R672, 1999

18. Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248-254, 1976

19. Towbin H, Staehelin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc Natl Acad Sci USA 76:4350-4354, 1979

20. Turcotte LP, Swenberger JR, Tucker MZ, et al: Training-induced elevation in FABPpm is associated with increased palmitate use in contracting muscle. J Appl Physiol 87:285-293, 1999

21. Burton GW, Webb A, Ingold KU: Methods: A mild, rapid and efficient method of lipid extraction for use in determining vitamin E/lipid ratios. Lipids 20:29-39, 1985

22. Wang JL, Chinookoswong N, Scully S, et al: Differential effects of leptin in regulation of tissue glucose utilization in vivo. Endocrinology 140:2117-2124, 1999

23. Brozinick JT Jr, Etgen GJ Jr, Yaspelkis BB III, et al: Effects of exercise training on muscle GLUT4 protein content and translocation in obese Zucker rats. Am J Physiol 265:E419-E427, 1993

24. Dela F, Mikines KJ, Linstow M, et al: Effect of training on insulin-mediated glucose uptake in human muscle. Am J Physiol 263: E1134-E1143, 1992

25. Reynolds TH IV, Brozinick JT Jr, Rogers MA, et al: Effects of exercise training on glucose transport and cell-surface GLUT-4 in isolated rat epitrochlearis muscle. Am J Physiol 274:E773-E778, 1997

26. Duronio V, Claffey KP, Ettinger S: Downstream signaling events regulated by phosphatidylinositol 3-kinase activity. Cell Signal 10:233-239, 1998

27. Kanai F, Ito K, Todaka M, et al: Insulin-stimulated GLUT4 translocation is relevant to the phosphorylation of IRS-1 and the activity of PI3-kinase. Biochem Biophys Res Commun 195:762-768, 1994

28. Ceddia RB, Koistinen HA, Zierath JR, et al: Analysis of paradoxical observations on the association between leptin and insulin resistance. FASEB J 16:1163-1176, 2002

29. Yeh J-I, Gulve EA, Rameh L, et al: The effects of wortmannin on rat skeletal muscle. J Biol Chem 270:2107-2111, 1995

30. Kellerer M, Koch M, Metzinger E, et al: Leptin activates PI-3 kinase in C_2C_{12} myotubes via janus kinase-2 (JAK-2) and insulin receptor substrate-2 (IRS-2) dependent pathways. Diabetologia 40: 1358-1362, 1997

31. Wilkes JJ, Bonen A, Bell RC: A modified high-fat diet induces insulin resistance in rat skeletal muscle but not adipocytes. Am J Physiol 275:E679-E686, 1998

32. Hansen PA, Han DH, Marshall BA, et al: A high fat diet impairs stimulation of glucose transport in muscle. Functional evaluation of potential mechanisms. J Biol Chem 273:26157-26163, 1998

33. Buettner R, Newgard CB, Rhodes CJ, et al: Correction of diet-induced hyperglycemia, hyperinsulinemia, and skeletal muscle insulin resistance by moderate hyperleptinemia. Am J Physiol 278: E563-E569, 2000

34. Halseth AE, Bracy DP, Wasserman DH: Limitation to basal and insulin-stimulated skeletal muscle glucose uptake in the high-fat-fed rat. Am J Physiol 279:E1064-E1071, 2000

35. Barnard RJ, Roberts CK, Varon SM, et al: Diet-induced insulin resistance precedes other aspects of the metabolic syndrome. J Appl Physiol 84:1311-1315, 1998

36. Kraegen EW, James DE, Storlein LH, et al: In vivo insulin resistance in individual peripheral tissues of the high fat fed rat: Assessment by euglycaemic clamp plus deoxyglucose administration. Diabetologia 29:192-198, 1986

37. Tremblay F, Lavigne C, Jacques H, et al: Defective insulin-induced GLUT4 translocation in skeletal muscle of high fat-fed rats is associated with alterations in both Akt/protein kinase B and atypical protein kinase C (ζ/λ) activities. Diabetes 50:1901-1910, 2001

38. Krook A, Bjornholm M, Galuska D, et al: Characterization of signal transduction and glucose transport in skeletal muscle from type 2 diabetic patients. Diabetes 49:284-292, 2000

39. Song XM, Kawano Y, Krook A, et al: Muscle fiber type-specific defects in insulin signal transduction to glucose transport in diabetic GK rats. Diabetes 48:664-670, 1999

40. Krook A, Roth RA, Jiang XJ, et al: Insulin-stimulated Akt kinase activity is reduced in skeletal muscle from NIDDM subjects. Diabetes 47:1281-1287, 1998

41. Zierath JR, Houseknecht KL, Gnudi L, et al: High-fat feeding impairs insulin-stimulated GLUT4 recruitment via an early insulin-signaling defect. Diabetes 46:215-223, 1997

42. Nadler ST, Stoehr JP, Rabaglia ME, et al: Normal Akt/PKB with reduced PI3K activation in insulin-resistant mice. Am J Physiol 281: E1249-E1254, 2001

43. Kim Y-B, Nikoulina SE, Ciaraldi TP, et al: Normal insulin-dependent activation of Akt/protein kinase B, with diminished activation of phosphoinositide 3-kinase, in muscle in type 2 diabetes. J Clin Invest 104:733-741, 1999

44. Cho H, Thorvaldsen JL, Chu Q, et al: Akt1/PKBα is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. J Biol Chem 276:38349-38352, 2001

45. Kruszynska YT, Worrall DS, Ofrecio J, et al: Fatty acid-induced insulin resistance: Decreased muscle PI3K activation but unchanged Akt phosphorylation. J Clin Endocrinol Metab 87:226-234, 2002

46. Barzilai N, She L, Liu L, et al: Decreased visceral adiposity accounts for leptin effect on hepatic but not peripheral insulin action. Am J Physiol 277:E291-E298, 1999

47. Muoio DM, Dohm GL, Fiedorek FT Jr, et al: Leptin directly alters lipid partitioning in skeletal muscle. Diabetes 46:1360-1363, 1997

48. Steinberg GR, Dyck DJ, Calles-Escandon J, et al: Chronic leptin treatment decreases fatty acid uptake and fatty acid transporters in rat skeletal muscle. J Biol Chem 277:8854-8860, 2002

49. Koyama K, Chen G, Lee Y, et al: Tissue triglycerides, insulin resistance, and insulin production: Implications for hyperinsulinemia of obesity. Am J Physiol 273:E708-E713, 1997

50. Takahashi N, Waelput W, Guisez Y: Leptin is an endogenous protective protein against the toxicity exerted by tumor necrosis factor. J Exp Med 189:207-212, 1999

51. Mito N, Hosoda T, Kato C, et al: Change of cytokine balance in diet-induced obese mice. Metabolism 49:1295-1300, 2000

52. Stephens JM, Pekala PH: Transcriptional repression of the GLUT4 and C/EBP genes in 3T3-L1 adipocytes by tumor necrosis factor-α. J Biol Chem 266:21839-21845, 1991

53. Hotamisligil GS, Spiegelman BM: Tumor necrosis factor α: A key component of the obesity-diabetes link. Diabetes 43:1271-1278, 1994

54. Del Aguila LF, Claffey KP, Kirwan JP: TNF-α impairs insulin signaling and insulin stimulation of glucose uptake in C_2C_{12} muscle cells. Am J Physiol 276:E849-E855, 1999

55. Paz K, Hemi R, LeRoith D, et al: A molecular basis for insulin resistance. J Biol Chem 272:29911-29918, 1997

56. Hotamisligl G, Peraldi P, Budavari A, et al: IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α–and obesity-induced insulin resistance. Science 271:665-668, 1996