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Exercise training increases components of the c-Cbl–associated protein/c-Cbl signaling cascade in muscle of obese Zucker rats

Jeffrey R. Bernard[a], Misato Saito[b], Yi-Hung Liao[a], Ben B. Yaspelkis III [b], John L. Ivy[a,*]

[a]*Exercise Physiology and Metabolism Laboratory, Department of Kinesiology and Health Education, University of Texas at Austin, Austin, TX 78712, USA*
[b]*Exercise Biochemistry Laboratory, Department of Kinesiology, California State University Northridge, Northridge, CA 91330, USA*

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Abstract

The purpose of this investigation was to determine whether alterations in the c-Cbl–associated protein/c-Cbl pathway and/or p38-mitogen–activated protein kinase (p38 MAP kinase) were associated with improved skeletal muscle insulin responsiveness in exercise-trained obese Zucker rats. Obese Zucker rats ran 5 d/wk on a motorized treadmill for 90 minutes over a 7-week period. Age-matched obese Zucker rats (OB-SED) and their lean littermates (LN-SED) were obtained to serve as nontrained controls. Twenty-four (OB-EX-24 h) or 48 hours (OB-EX-48 h) after the last exercise bout, the trained rats were studied via the hind limb perfusion technique in the presence of insulin. Insulin-stimulated glucose uptake was significantly decreased across the skeletal muscle of OB-SED rats compared with LN-SED, but was normalized in the obese rats by 7 weeks of training. The insulin-stimulated plasma membrane protein concentrations of TC10 and glucose transporter 4 were reduced in the sedentary Zuckers, but both proteins were increased by the training protocol. Training did not increase insulin-stimulated p38 MAP kinase protein concentration, nor did it have an effect on insulin-stimulated p38 MAP kinase phosphorylation at the plasma membrane. These results suggest that skeletal muscle insulin resistance is associated with reduced expression of TC10 and that this deficiency can be corrected with exercise training.

1. Introduction

Skeletal muscle of the obese Zucker rat is characterized by severe insulin resistance due to a combination of reduced insulin sensitivity and responsiveness [1,2]. Compared with lean littermates, obese Zucker rats have a reduced number of insulin receptors at the plasma membrane [3,4] and a defect in the insulin-stimulated glucose transporter 4 (GLUT4) translocation process [2,5,6]. However, it is well established that aerobic training is an effective exercise modality for the prevention and reversal of skeletal muscle insulin resistance and has been shown to improve insulin-stimulated glucose transport in the obese Zucker rat [1,7]. Christ et al [8] observed increased insulin-stimulated glucose uptake in obese Zucker rats trained for 7 weeks by treadmill running. Although skeletal muscle insulin resistance was improved in the trained animals, as evidenced by increased insulin-stimulated glucose uptake, this did not appear to be due to alterations in signaling proteins acting through the phosphatidylinositol 3-kinase (PI 3-kinase) pathway. These findings suggest that the adaptations to exercise training in obese Zucker rats may be due to an alternate signaling pathway.

Recently, it has been demonstrated in both adipose tissue [9] and L6 myotubes [10] that c-Cbl and c-Cbl–associated protein (CAP) are signaling proteins involved in insulin-stimulated glucose uptake that are independent of PI 3-kinase activity. In the presence of insulin, the adaptor protein APS (adaptor containing PH and SH2 domains) recruits the CAP/c-Cbl complex to the insulin receptor for the tyrosine phosphorylation of c-Cbl [11-13]. The activated CAP/c-Cbl protein complex dissociates from the receptor and is targeted to the membrane-bound protein flotillin located at the lipid raft subdomains [11-13]. It is in the lipid rafts where the adaptor protein CAP binds to flotillin and c-Cbl tyrosine phosphorylates the CrkII/C3G complex. C3G

* Corresponding author. Department of Kinesiology and Health Education, Bellmont Hall 222, The Univ. of Texas at Austin, Austin, TX 78712-0360, USA.
E-mail address: johnivy@mail.utexas.edu (J.L. Ivy).

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acts as a guanine nucleotide exchange factor for the Rho family member TC10. The activation of the small guanosine triphosphate (GTP)-binding protein TC10 facilitates insulin-stimulated guanosine triphosphate GLUT4 translocation to the plasma membrane [9,14,15].

Although the relative importance of the CAP/c-Cbl signaling pathway in skeletal muscle glucose uptake is unclear, there have been studies conducted to investigate the effect of exercise training on this signaling pathway. Bernard et al [16] found that plasma membrane–associated c-Cbl content was unchanged by 12 weeks of treadmill running in Sprague-Dawley skeletal muscle, but observed a significant increase in c-Cbl tyrosine phosphorylation. To the best of our knowledge, there has been no investigation of exercise training on the CAP/c-Cbl pathway in the muscle of insulin-resistant obese Zucker rats, although Wadley et al [4] found no change in c-Cbl protein concentration or phosphorylation status with an acute bout of exercise. Thus, the first aim of the present study was to investigate whether the improved insulin resistance in obese Zucker rats after 7 weeks of exercise training was associated with alterations in the CAP/c-Cbl signaling pathway.

Altered p38-mitogen–activated protein kinase (p38 MAP kinase) activity is another possible explanation for improved insulin-stimulated muscle glucose uptake post exercise training in the obese Zucker rat. It has been suggested that p38 MAP kinase may play a role in activating GLUT4 for glucose transport. Although the cause-and-effect relationship of p38 MAP kinase and GLUT4 activation is unclear, it is noteworthy that p38 MAP kinase appears to be activated by both insulin and muscle contraction [17]. Interestingly, it has been shown that inhibiting p38 MAP kinase activity prevented the activation but not the translocation of GLUT4 to the plasma membrane [18]. In addition, blocking p38 MAP kinase had no effect on the PI 3-kinase signaling pathway under insulin-stimulated conditions [18]. Thus, a secondary aim of this investigation was to investigate if p38 MAP kinase adapted to 7 weeks of treadmill running and if alterations to p38 MAP kinase were associated with improved insulin resistance in the trained obese Zucker rat.

2. Materials and methods

2.1. Experimental design

Animal characteristics and the training protocol for this study have been previously reported [8,19]. Two groups of 7-week–old female obese Zucker rats (OB-EX-24 h and OB-EX-48 h, n = 7 each) were trained for 5 d/wk for 7 weeks on a motorized treadmill. Rats initially ran at 15 m/min for 10 minutes on an 8% grade. Over the proceeding 6 weeks, the work rate was gradually increased until they could run continuously at 22 m/min for 90 minutes on an 8% grade. This exercise protocol was maintained for the duration of training. On the last day of training, rats were subjected to an additional 10-minute exercise bout at 26 m/min after a 5-minute rest period. All rats were 14 weeks of age at the end of training. Fourteen-week–old female obese Zucker rats (OB-SED, n = 8) and their female lean littermates (LN-SED, n = 8) were obtained to serve as nontrained controls. All rats were housed 3 to a cage and provided standard laboratory chow and water ad libitum. The temperature of the animal room was maintained at 21°C, and an artificial 12-hour/12-hour light-dark cycle was set. Food was withdrawn 12 to 14 hours before hind limb perfusion. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Texas at Austin.

2.2. Surgical preparation and hind limb perfusions

After an overnight fast, all rats were subjected to the hind limb perfusion technique. The training groups were subjected to the hind limb perfusion 24 or 48 hours after their last exercise bout. Details for the surgical preparation and hind limb perfusion technique have been described elsewhere [8,19]. Perfusate flow was limited to the right hind limb of the rat by cannulating the right iliac artery and vein to the tip of the femoral artery. The right hind limb was then washed out with 35 mL of Krebs-Henseleit buffer. After the washout period, the rat was euthanized by a cardiac injection of sodium pentobarbital. Cannulas were then placed in line with the perfusion system, and the hind limb was allowed to stabilize during a 10-minute nonrecirculating washout period. The perfusion medium consisted of Krebs-Henseleit buffer (pH 7.4) containing 4.5% dialyzed bovine serum, 20% washed time-expired human red blood cells, 1 mmol/L glucose, 10 μU/mL Humulin (Lilly, Indianapolis, IN), and 0.2 mmol/L pyruvate. The perfusion flow rate during the washout period was set at 5 mL/min for 10 minutes. After the washout period, the arterial line was switched to perfusate containing 10 mU/mL Humulin, 6 mmol/L glucose, 0.2 mmol/L pyruvate, 2 mmol/L mannitol, 0.2 μCi/mL 2-[^{3}H] deoxyglucose (2-[^{3}H] DG), 0.15 μCi/mL [^{14}C] mannitol, and the same concentration of human red blood cells and bovine serum albumin used during the washout period. The perfusions were performed at 37°C and continued for a total of 22 minutes, at which time the right gastrocnemius and hamstring muscles were excised, freeze clamped in liquid nitrogen, and stored at −80°C for later analysis.

Determination of skeletal muscle glucose uptake

Rates of 2-[^{3}H] DG uptake were determined from the plantaris muscle as described previously [8]. Muscle samples were weighed, homogenized in 1 mL of 10% trichloroacetic acid, and centrifuged for 10 minutes at 4°C. From the supernatant, 0.3 mL was transferred to scintillation vials containing 10 mL of Scintiverse E (Fisher Scientific, Pittsburgh, PA). Well-mixed arterial perfusate samples were taken during the hind limb perfusion and treated the same as the muscle homogenate. All samples were counted

for radioactivity in an LS-350 liquid scintillation spectrophotometer (Beckman, Fullerton, CA). The accumulation of 2-[^{3}H] DG in the intracellular space was used to indicate muscle glucose uptake. Extracellular accumulation of 2-[^{3}H] DG in the extracellular space was assessed by measuring the concentration of [^{14}C] mannitol in the muscle homogenate. Glucose uptake across the gastrocnemius was then calculated by subtracting the concentration of 2-[^{3}H] DG in the extracellular space from the total muscle 2-[^{3}H] DG concentration.

2.4. Citrate synthase activity

To demonstrate that there was a training response of the hamstring muscle, the activity of citrate synthase was assessed. Citrate synthase activity was determined spectrophotometrically according to Srere [20].

2.5. Plasma membrane fractionation

Plasma membrane fractions were prepared from insulin-stimulated hamstring muscle samples as described previously [21,22]. Briefly, muscle sample was diluted 1:7 in 10 mmol/L Tris–15% sucrose solution (pH 7.5) that contained 0.1 mmol/L phenylmethylsulfonyl fluoride, 10 mmol/L ethylene glycol tetraacetic acid (EGTA), and 10 mg/mL trypsin inhibitor and homogenized. The homogenate was filtered and centrifuged at 100 000*g* for 1 hour. The pellet was resuspended in 10 mmol/L Tris–15% sucrose buffer, and a small aliquot from this resuspension was collected as the crude homogenate for later analysis. The remaining homogenate was layered on continuous sucrose gradients (35%-70%) and centrifuged at 120 000*g* for 2 hours. The plasma membrane layer was collected, washed in 10 mmol/L Tris buffer, and centrifuged for 1 hour at 100 000*g*. The final plasma membrane pellet was resuspended in 200 μL/g of original tissue of a homogenization buffer (50 mmol/L N-[2-hydroxyethyl] piperazine-N′-[2-ethane-sulfonic acid [HEPES], 150 mmol/L NaCl, 200 mmol/L sodium pyrophosphate, 20 mmol/L β-glycerolphosphate, 20 mmol/L NaF, 2 mmol/L sodium vanadate, 20 mmol/L EDTA, 1% octylphenyl-polyethylene glycol [IGEPAL], 10% glycerol, 2 mmol/L phenylmethylsufonyl fluoride, 1 mmol/L $MgCl_2$, 1 mmol/L $CaCl_2$, 10 μg/mL leupeptin, and 10 μg/mL aprotinin) and stored at −80°C for later analysis.

To ensure that purified plasma membrane fractions were being used for analysis, the activity of the membrane marker enzyme 5′-nucleotidase was assessed in plasma membrane fractions and compared with the activity in crude homogenate fractions.

Ten microliters of plasma membrane or crude homogenate fractions was added to 200 μL of a reaction cocktail (0.5 mol/L glycine, 0.01 mol/L $MgCl_2$, and 0.005 mol/L adenosine monophosphate) and incubated at 37°C for 45 minutes. The reaction was stopped by the addition of 8% trichloroacetic acid, and the samples were centrifuged at 25 000 rpm for 10 minutes. The supernatant was then added to a tube containing 200 μL acid molybdate and 300 μL of deionized water. Fiske and SubbaRow solution (Sigma Diagnostics, St Louis, MO) was added to each sample, which was incubated at room temperature (RT) for 10 minutes; and the activity of 5′-nucleotidase was determined spectrophotometrically. The specific activities, purity index, and percentage yield of the plasma membrane marker 5′-nucleotidase were determined before detection of proteins in the plasma membrane fraction (Table 1).

Table 1
Characteristics of 5′-nucleotidase activity in the crude homogenate and plasma membrane

	LN-SED	OB-SED	OB-EX-24 h	OB-EX-48 h
5′-Nucleotidase activity (μmol/[mg min])				
Cr	4.1 ± 0.2	4.8 ± 0.3	5.5 ± 0.3	5.6 ± 0.3
PM	13.9 ± 1.4 *	17.1 ± 1.3 *	15.6 ± 0.3 *	17.2 ± 1.1 *
Purity index	3.7 ± 0.4	2.9 ± 0.1	3.1 ± 0.2	3.4 ± 0.3
% Yield	17.4 ± 1.7	20.2 ± 2	17.9 ± 1.3	19.3 ± 1.1

Values are means ± SE. Cr indicates crude homogenate; PM, plasma membrane.
* $P < .05$ significantly different from Cr.

2.6. p38 MAP kinase protein concentration and phosphorylation

p38 MAP kinase protein concentration and phosphorylation status were determined from insulin-stimulated plasma membrane and crude homogenate fractions obtained from the hamstring. Sample protein (100 μg) was subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and the proteins were separated on a 10% resolving gel. The resolved proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using a semidry transfer unit and blocked in 5% nonfat dry milk in Tris-Tween–buffered saline (NFDM/TTBS) for 1.5 hours at RT. The membranes were then incubated with either affinity-purified anti-p38 MAP kinase (catalog no. 9212; Cell Signaling Technology, Danvers, MA) or phospho-p38 MAP kinase (catalog no. 9211, Cell Signaling Technology) overnight at 4°C. After three 5-minute washes in TTBS, the membranes were incubated for 1 hour at RT with the species-specific immunoglobulin G (IgG) secondary antibodies. The membranes were washed 3 times in TTBS, and antibody binding was visualized on x-ray film by enhanced chemiluminescence in accordance to the manufacturer's instructions (West Femto; Pierce Chemical, Rockford, IL). The images were digitized by scanning, and the density of the bands was quantified as a percentage of a standard run on each gel using Image J software (National Institute of Health, Bethesda, MD).

2.7. Plasma membrane CAP and TC10 protein concentrations

The CAP and TC10 protein concentrations were determined from insulin-stimulated plasma membrane fractions obtained from the hamstring. Sample protein (200 μg

for CAP and 250 μg for TC10) was incubated with anti-CAP (catalog no. 06-994; Upstate Biotechnology [UBT], Charlottesville, VA) or anti-TC10 (catalog no. sc-12637; Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C. After the overnight incubation, 100 μL of protein A–sepharose slurry (Amersham Pharmacia Biotechnology, Piscataway, NJ) was added to the immunoprecipitates and rotated for 1.5 hour at 4°C. The immunocomplexes were then washed as previously described [23]. After the last wash, 20 μL of a 1:1 solution of Laemmli sample buffer (125 mmol/L Tris, 20% glycerol, 2% SDS, 0.008% bromophenol blue, pH 6.8) and homogenization buffer was added to each sample, boiled for 5 minutes at 100°C, and centrifuged for 20 seconds in a minicentrifuge. Ten microliters of supernatant was subjected to SDS-PAGE run on either a 7.5% (CAP) or 12.5% (TC10) resolving gel. The resolved proteins were transferred to a PVDF membrane and blocked in 5% NFDM/TTBS. Membranes were incubated with affinity-purified anti-CAP (catalog no. 06-994, UBT) or anti-TC10 (catalog no. sc-12637, Santa Cruz Biotechnology), followed by the species-specific IgG secondary antibody. Antibody binding was visualized by enhanced chemiluminescence in accordance to the manufacturer's instructions (West Femto, Pierce Chemical). Images were captured using a charge-coupled device camera in a ChemiDoc system (BioRad, Hercules, CA) and saved to a Macintosh G4 (Apple, Cupertino, CA) computer. The density of the bands was quantified with a Quantity One analysis software (BioRad) and expressed as a percentage of a standard run on each gel.

2.8. Plasma membrane c-Cbl, flotillin, and GLUT4 protein concentrations

The protein concentrations of c-Cbl, flotillin, and GLUT4 were determined from insulin-stimulated plasma membrane fractions obtained from hamstring samples. Seventy micrograms of sample protein was subjected to SDS-PAGE run under reducing conditions on either a 7.5% (c-Cbl) or 10% (flotillin and GLUT4) resolving gel and transferred to a PVDF membrane. The membranes were blocked in 5% NFDM/TTBS and incubated with either affinity-purified anti–c-Cbl (catalog no. 05-440, UBT), anti–flotillin-1 (catalog no. 610820; BD Transduction Laboratories, BD Biosciences Pharmingen, San Diego, CA), or anti-GLUT4 (donated by Dr Samuel Cushman, National Institute of Diabetes and Digestive and Kidney Disease, Bethesda, MD) antibodies followed by the species-specific IgG secondary antibody. Antibody binding was visualized and quantified as described above.

2.9. Statistical analysis

A 1-way analysis of variance was performed on all data sets. When a significant F ratio was obtained, a least significant difference post hoc test was performed to identify statistically significant differences ($P < .05$) between means. All statistical analyses were completed using SPSS software (SPSS, Chicago, IL), and all values were expressed as means ± standard error (SE).

3. Results

3.1. Animal characteristics

The physical characteristics of these animals have been reported previously [8,19]. The OB-SED rats were significantly heavier than their lean littermates, LN-SED (373.1 ± 4.8 vs 200.1 ± 4.5 g). Treadmill running did not significantly reduce the body weight of the OB-EX-24 h (356.1 ± 3.2 g) or OB-EX-48 h (359.1 ± 5.2 g) rats. The LN-SED rats were significantly lighter than all treatment groups.

3.2. Muscle glucose uptake

Rates of insulin-stimulated 2-[^{3}H] DG uptake were reduced in OB-SED compared with LN-SED controls (Table 2). Seven weeks of treadmill running significantly increased insulin-stimulated glucose uptake in the obese Zucker rats; however, both OB-EX-24 h and OB-EX-48 h were still significantly lower than that of LN-SED. There was no difference in glucose uptake between OB-EX-24 h and OB-EX-48 h.

3.3. Citrate synthase activity

Citrate synthase activity was significantly elevated in the skeletal muscle of all obese groups compared with the lean sedentary group (Table 2). Seven weeks of exercise training increased citrate synthase activity in the obese trained groups. This increase in activity was observed both 24 and 48 hours after the last exercise bout. However, there was no statistically significant difference between OB-EX-24 h and OB-EX-48 h rats.

Table 2
Citrate synthase activity and rates of glucose uptake

	LN-SED	OB-SED	OB-EX-24 h	OB-EX-48 h
Citrate synthase (μmol/[g min])	20.1 ± 1.2	31.7 ± 1.2 *	44.7 ± 3.1 *, **	54.0 ± 3.5 *, **
Glucose uptake (mmol/[h g])	7.0 ± 0.53	2.2 ± 0.25 ***	5.5 ± 0.48 *	5.3 ± 0.57 *

Values are means ± SE.
* $P <$ vs LN-SED.
** $P < .05$, significantly different from OB-SED.
*** $P < .05$, significantly different from all groups.

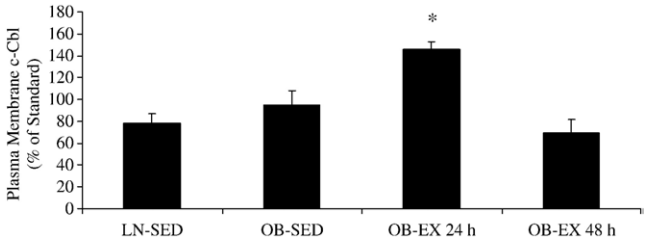

Fig. 1. Plasma membrane c-Cbl protein concentration obtained from the insulin-stimulated hamstring of LN-SED (n = 8), OB-SED (n = 8), OB-EX-24 h (n = 7), or OB-EX-48 h (n = 7) animals. Values are expressed as means ± SE. *$P < .05$, significantly different from all groups.

3.4. CAP/c-Cbl signaling pathway

The determination of c-Cbl, CAP, flotillin, and TC10 was limited to the plasma membrane in the presence of insulin. We were previously unable to detect protein concentrations of c-Cbl and CAP [16] as well as flotillin and TC10 (unpublished observations) in the crude fraction under both insulin- and non–insulin-stimulated conditions. In contrast, these proteins were readily detectable in the plasma membrane fractions, suggesting that components of the novel insulin signaling cascade are localized to the plasma membrane. In the presence of insulin, plasma membrane c-Cbl protein concentration was not different between sedentary lean and obese littermates (Fig. 1). Although 7 weeks of treadmill running significantly increased c-Cbl protein concentration, this elevation was not sustained 48 hours after the last exercise bout. Similar to c-Cbl, CAP protein concentration was not different between LN-SED and OB-SED rats (Fig. 2). Training significantly increased the plasma membrane concentration of CAP, and this adaptation appears to persist for at least 48 hours. The protein concentration of TC10 was significantly decreased at the plasma membrane of obese sedentary rats (Fig. 3). This reduction appeared to be normalized with 7 weeks of treadmill running in the OB-EX-24 h and OB-EX-48 h rats. No differences were found among treatment groups for the membrane-bound protein flotillin (Fig. 4).

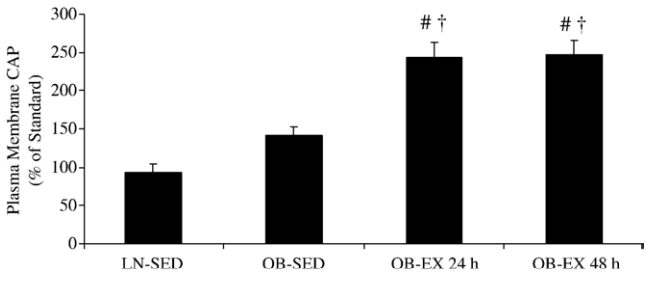

Fig. 2. Plasma membrane CAP protein concentration obtained from the insulin-stimulated hamstring of LN-SED (n = 8), OB-SED (n = 8), OB-EX-24 h (n = 7), or OB-EX-48 h (n = 7) animals. Values are expressed as means ± SE. #$P < .05$, significantly different from LN-SED; †$P < .05$, significantly different from OB-SED.

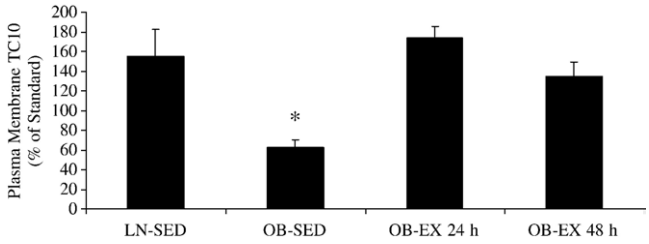

Fig. 3. Plasma membrane TC10 protein concentration obtained from the insulin-stimulated hamstring of LN-SED (n = 8), OB-SED (n = 8), OB-EX-24 h (n = 7), or OB-EX-48 h (n = 7) animals. Values are expressed as means ± SE. *$P < .05$, significantly different from all groups.

3.5. GLUT4 protein concentration

Insulin stimulation resulted in a significant elevation in plasma membrane GLUT4 protein concentration across treatment groups (Fig. 5). However, the number of glucose transporters associated with the plasma membrane was significantly reduced in the OB-SED rats when compared with LN-SED. Seven weeks of treadmill running increased insulin-stimulated plasma membrane GLUT4 protein concentration in OB-EX-24 h and OB-EX-48 h compared with OB-SED rats. The concentration of the glucose transporter at the plasma membrane was significantly increased in OB-EX-24 h compared with LN-SED as well. Although plasma membrane GLUT4 protein concentration 48 hours after the last bout of exercise for OB-EX-48 h remained elevated above OB-SED rats in the presence of insulin, the GLUT4 protein concentration was significantly below that of OB-EX-24 h rats. In addition, there was no statistical difference in GLUT4 protein between LN-SED and OB-EX-48 h rats.

3.6. p38 MAP kinase protein concentration and phosphorylation

Total p38 MAP kinase protein concentration was not different among all the groups in the crude homogenate under insulin-stimulated conditions (Fig. 6). In addition, there was no statistical difference in insulin-stimulated plasma membrane p38 MAP kinase protein concentration. However, the protein concentration was significantly elevated in the plasma membrane compared with the crude fraction for all groups.

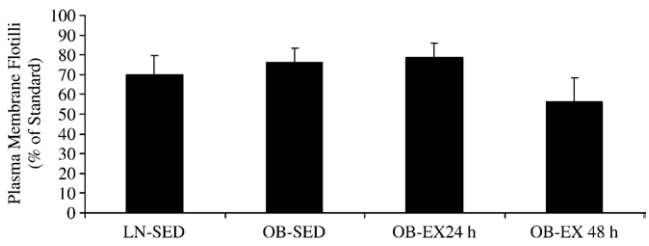

Fig. 4. Plasma membrane flotillin protein concentration obtained from the insulin-stimulated hamstring of LN-SED (n = 8), OB-SED (n = 8), OB-EX-24 h (n = 7), or OB-EX-48 h (n = 7) animals. Values are expressed as means ± SE.

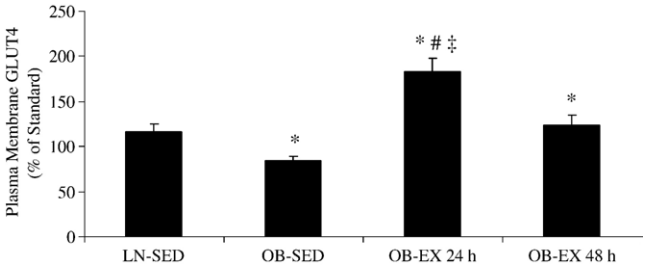

Fig. 5. Plasma membrane GLUT4 protein concentration obtained from the insulin-stimulated hamstring of LN-SED (n = 8), OB-SED (n = 8), OB-EX-24 h (n = 7), or OB-EX-48 h (n = 7) animals. Values are expressed as means ± SE. *$P < .05$, significantly different from all groups; #$P < .05$, significantly different from LN-SED; ‡$P < .05$, significantly different from OB-EX-48 h.

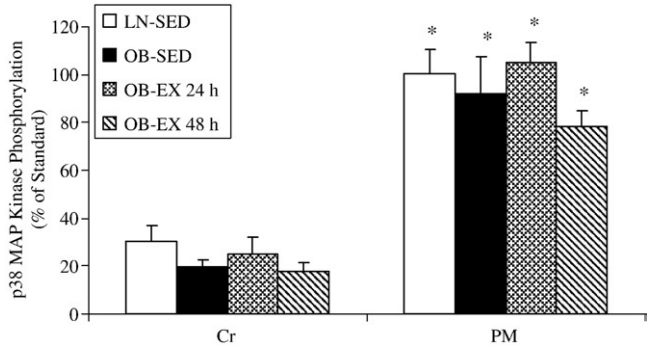

Fig. 7. p38 MAP kinase phosphorylation obtained from the insulin-stimulated hamstring of LN-SED (n = 8), OB-SED (n = 8), OB-EX-24 h (n = 7), or OB-EX-48 h (n = 7) animals. Values are expressed as means ± SE. *$P < .05$, significantly different from all crude homogenate groups.

Similar to total p38 MAP kinase protein concentration, p38 MAP kinase phosphorylation for insulin-stimulated plasma membrane fractions was elevated above their respective crude fraction (Fig. 7). No differences existed among insulin-stimulated plasma membrane treatment groups.

4. Discussion

Exercise training is recognized as an effective prescription for both the prevention and reversal of insulin resistance. The action of exercise training appears to be exerted by increased insulin responsiveness of skeletal muscle [7]. Although exercise training increases insulin-stimulated glucose uptake in the obese Zucker rat, it has been shown previously that this is not due to improved insulin signaling through the PI 3-kinase pathway [8]. Because the increased skeletal muscle responsiveness after 7 weeks of treadmill running in Zucker rats could not be explained by alterations through the PI 3-kinase pathway, Christ et al [8] suggested that either an alternate pathway, such as the CAP/c-Cbl (novel) cascade, or p38 MAP kinase may be involved.

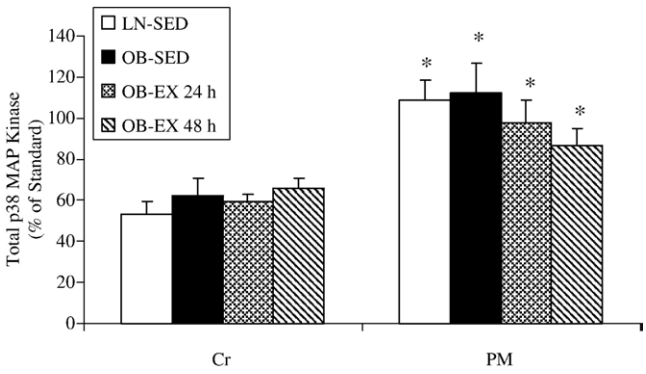

Fig. 6. Total p38 MAP kinase protein concentration obtained from the insulin-stimulated hamstring of LN-SED (n = 8), OB-SED (n = 8), OB-EX-24 h (n = 7), or OB-EX-48 h (n = 7) animals. Values are expressed as means ± SE. *$P < .05$, significantly different from all crude homogenate groups.

Therefore, the purpose of the present investigation was to determine whether alterations in the CAP/c-Cbl pathway and/or p38 MAP kinase were associated with improved skeletal muscle insulin resistance in obese Zucker rats.

Despite the fact that the CAP/c-Cbl pathway does exist in skeletal muscle, its role in insulin-stimulated glucose transport has been debated [24]. However, studies have demonstrated alterations of this pathway in rodent skeletal muscle in response to high-fat feeding [21], monosodium glutamate treatment [25], streptozotocin treatment [26], and exercise [4,16]. In the present investigation, we report no difference in the plasma membrane concentration of c-Cbl, CAP, or flotillin among sedentary lean and obese Zucker rats after insulin stimulation. This agrees with the results presented by Wadley et al [4] who found no difference in c-Cbl protein concentration among the homogenates of lean and obese Zucker rats. In comparing healthy Sprague-Dawley muscle with those fed a high-fat diet to induce insulin resistance, there was also no difference in plasma membrane protein concentration observed for c-Cbl, CAP, and flotillin [21]. Collectively, these findings would suggest that reduced glucose clearance in insulin-resistant skeletal muscle is not associated with reductions in the protein expression of proximal components of this novel cascade.

Although proximal components of the novel cascade may not be impaired in insulin-resistant skeletal muscle, we did find a significant reduction in plasma membrane TC10 protein concentration. In contrast, insulin-stimulated TC10 protein concentration was not decreased in high-fat–fed Sprague-Dawley rats [16]. These differences could be attributed to the model of skeletal insulin resistance used. Nevertheless, TC10 may represent a primary defect through the CAP/c-Cbl pathway for insulin receptor signaling in obese Zucker rats. This is an intriguing possibility because it has been suggested that TC10 mediates cortical actin remodeling for GLUT4 translocation in adipocytes [27]. It is well established that the novel pathway is required for insulin-stimulated GLUT4 translocation in adipocytes [15] and suggests that activation of TC10 is required for insulin-

stimulated adipocyte glucose uptake [28]. Impairing the insulin receptor signal at TC10 by knockdown of the TC10 α isoform was recently shown by Chang et al [28] to inhibit GLUT4 translocation and insulin-stimulated glucose uptake in adipocytes. Thus, it is feasible that TC10 serves a similar purpose in skeletal muscle and that decreased TC10 protein expression may be involved in defective insulin-stimulated GLUT4 translocation in sedentary obese Zucker rats. However, Gupte and Mora [26] found that despite the activation of c-Cbl, TC10 was not activated in insulin-stimulated skeletal muscle, suggesting that the role of TC10 may differ in adipocytes and skeletal muscle.

There is a paucity of studies in the literature that have investigated the effects of exercise on the CAP/c-Cbl signaling pathway. Those that have studied this pathway under exercise conditions have done so after an acute bout of exercise in insulin-resistant tissue [4] or after training in healthy rodent skeletal muscle [16]. Thus, this appears to be the first study to measure components of the CAP/-c-Cbl pathway in the skeletal muscle of exercise-trained obese Zucker rats. Wadley et al [4] measured c-Cbl protein and phosphorylation status in lean and obese Zucker rats, but this was performed in whole muscle homogenates after a single bout of exhaustive exercise. Although Bernard et al [16] assessed c-Cbl protein and phosphorylation at the plasma membrane after exercise training, this was measured in Sprague-Dawley skeletal muscle. For the present investigation, we limited our evaluation of the CAP/c-Cbl pathway in obese Zucker rats to the plasma membrane under insulin-stimulated conditions. Therefore, few direct comparisons can be made.

The present investigation found that insulin-stimulated plasma membrane c-Cbl, CAP, and TC10 protein concentrations were all increased by 7 weeks of exercise training. These changes were associated with an increase in the number of GLUT4 transporters at the plasma membrane 24 and 48 hours after the last bout of exercise in the presence of insulin. It is well established that there is a strong correlation between elevated plasma membrane GLUT4 and enhanced glucose transport [6] in obese Zucker rats under insulin-stimulated conditions. These results therefore suggest that the novel pathway may be capable of promoting insulin-stimulated GLUT4 translocation in skeletal muscle. Of interest, we found that plasma membrane TC10 expression, which was significantly reduced in the obese sedentary Zucker, was normalized by exercise training. This would then suggest that TC10, rather than proximal components of the novel cascade, is associated with the improved skeletal muscle insulin resistance in exercise-trained obese Zucker rats. Although the possible mechanism for these positive effects on skeletal muscle glucose clearance cannot be determined from the present data or from the literature at this time, it can be hypothesized that increases in the protein expression of the novel pathway, specifically TC10, enhance cortical actin remodeling, promoting GLUT4 translocation to the plasma membrane.

Our findings are in contrast to that previously observed in exercised insulin-resistant and healthy rodent skeletal muscle. After an acute bout of exercise, Wadley et al [4] found no change in the whole cell protein concentration of c-Cbl in the obese Zucker rats, suggesting that multiple bouts may be required to stimulate an increased expression of these proteins. However, Bernard et al [16] could not demonstrate an increase in the novel cascade proteins after 12 weeks of exercise training in Sprague-Dawley rats, although they did report that insulin-stimulated c-Cbl phosphorylation was significantly increased at the plasma membrane in exercise-trained Sprague-Dawley rats. The present investigation did not determine the phosphorylation status of the CAP/c-Cbl signaling proteins in exercise-trained obese Zucker rats. Thus, further studies are warranted to differentiate the significance of exercise-induced increases in c-Cbl protein concentration and its phosphorylation status. Such information may prove more valuable in assessing the role of this pathway under both exercise and insulin-stimulated conditions.

Recent evidence suggests that GLUT4 translocation and glucose transport are separate events. The movement of GLUT4 from an intracellular vesicular network to the plasma membrane and the subsequent transport of glucose across this membrane may involve the exocytosis of GLUT4, cortical actin remolding, and activation of GLUT4 at the plasma membrane. This coordinated process is facilitated by possibly 3 distinct insulin receptor signaling pathways: the activation of PI 3-kinase and Akt, CAP/c-Cbl activation of TC10, and p38 MAP kinase. Upon insulin stimulation, Akt is required for exocytosis of GLUT4 [29], TC10-mediated cortical actin remodeling is required for the translocation of GLUT4 [27], and p38 MAP kinase may be needed for transporter activation at the plasma membrane [30]. Therefore, a secondary purpose of the present investigation was to assess p38 MAP kinase after exercise training in obese Zucker rats.

Previous studies have demonstrated that the MAP kinase pathway is activated by exercise in both human [31,32] and rodent skeletal muscle [19,33,34]. However, this is the first study to investigate p38 MAP kinase after exercise training in the insulin-resistant obese Zucker rat. The present investigation found that p38 MAP kinase is more abundant at the plasma membrane compared with the crude homogenate, as there was a significantly greater protein concentration under insulin-stimulated conditions. In addition, there appeared to be no difference in plasma membrane protein concentration between lean and obese Zucker rats. Exercise training did not increase insulin-stimulated plasma membrane p38 MAP kinase protein concentration. Although not statistically significant, there was a trend showing reduced p38 MAP kinase concentration 24 and 48 hours after the final training bout. Using Sprague-Dawley rats, Lee et al [34] found that whole cell p38 MAP kinase concentration was increased after a short-term bout of exercise, but decreased 48 hours after completing 4 weeks of training.

In agreement with previous studies, we observed p38 MAP kinase phosphorylation under insulin-stimulated conditions [17,35]. However, there was no difference in insulin-stimulated phosphorylation between sedentary and exercise-trained groups. Previous studies in human [35] and rodent [34] skeletal muscle have shown p38 phosphorylation to be increased with short-term exercise. According to Thong et al [35], phosphorylation may be increased for up to 3 hours post exercise. The present data would suggest that phosphorylation may remain increased compared with the sedentary obese rats for up to 24 hours post exercise training, but return to baseline levels by 48 hours. It is noteworthy, however, that recently it was suggested that although increased p38 MAP kinase activity is associated with increased skeletal muscle insulin sensitivity, it may not be a required alteration for this enhancement after muscle contraction [36]. In addition, Turban et al [37] did not observe increased insulin-stimulated p38 MAP kinase phosphorylation compared with basal and reported no difference in insulin-stimulated or basal glucose uptake in the skeletal muscle of p38β MAP kinase null mice. Collectively, these findings suggest that this kinase may not play a role in the activation of GLUT4. Thus, further studies are required to determine the cause-and-effect relationship between GLUT4 activity and p38 MAP kinase.

In summary, the present investigation found that the insulin-stimulated protein concentrations of c-Cbl, CAP, and flotillin are similar between sedentary lean and obese Zucker rats. In contrast, the expression of TC10 at the plasma membrane was significantly reduced in the sedentary obese Zuckers. We report that unlike the PI 3-kinase pathway, insulin receptor signaling through the CAP/c-Cbl pathway is enhanced by exercise training in the obese Zucker rat. Seven weeks of exercise training increased insulin-stimulated plasma membrane c-Cbl, CAP, and GLUT4 protein concentrations and appeared to correct the defect in the protein concentration of TC10, suggesting that alterations in TC10, rather than proximal components of the novel cascade, are associated with improved insulin responsiveness. In addition, exercise and insulin appeared to have no effect on the plasma membrane–associated p38 MAP kinase protein concentration. However, both insulin and exercise training will increase p38 MAP kinase phosphorylation. In conclusion, the present results suggest, but do not conclusively prove, that the characteristic skeletal muscle insulin resistance of obese Zucker rats is due in part to a reduced expression of TC10. This defect, however, appears to be corrected with 7 weeks of exercise training.

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