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Resistance training enhances components of the insulin signaling cascade in normal and high-fat-fed rodent skeletal muscle

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Krisan, Adam D., Dale E. Collins, Andrew M. Crain, Connie C. Kwong, Mohenish K. Singh, Jeffrey R. Bernard, and Ben B. Yaspelkis III. Resistance training enhances components of the insulin signaling cascade in normal and high-fat-fed rodent skeletal muscle. *J Appl Physiol* 96: 1691–1700, 2004. First published January 5, 2004; 10.1152/japplphysiol.01054.2003.—Our laboratory recently reported that chronic resistance training (RT) improved insulin-stimulated glucose transport in normal rodent skeletal muscle, owing, in part, to increased GLUT-4 protein concentration (Yaspelkis BB III, Singh MK, Trevino B, Krisan AD, and Collins DE. *Acta Physiol Scand* 175: 315–323, 2002). However, it remained to be determined whether these improvements resulted from alterations in the insulin signaling cascade as well. In addition, the possibility existed that RT might improve skeletal muscle insulin resistance. Thirty-two male Sprague-Dawley rats were assigned to four groups: control diet (Con)-sedentary (Sed); Con-RT; high-fat diet (HF)-Sed; and HF-RT. Animals consumed their respective diets for 9 wk; then RT animals performed 12 wk of training (3 sets, 10 repetitions at 75% one-repetition maximum, 3×/wk). Animals remained on their dietary treatments over the 12-wk period. After the training period, animals were subjected to hindlimb perfusions. Insulin-stimulated insulin receptor substrate-1-associated phosphatidylinositol-3 kinase activity was enhanced in the red gastrocnemius and quadriceps of Con-RT and HF-RT animals. Atypical PKC-ζ/λ and Akt activities were reduced in HF-Sed and normalized in HF-RT animals. Resistance training increased GLUT-4 protein concentration in red gastrocnemius and quadriceps of Con-RT and HF-RT animals. No differences were observed in total protein concentrations of insulin receptor substrate-1, Akt, atypical PKC-ζ/λ, or phosphorylation of Akt. Collectively, these findings suggest that resistance training increases insulin-stimulated carbohydrate metabolism in normal skeletal muscle and reverses high-fat diet-induced skeletal muscle insulin resistance by altering components of both the insulin signaling cascade and glucose transporter effector system.

high-fat diet; insulin resistance; phosphatidylinositol 3-kinase activity; atypical protein kinase C-ζ/λ; insulin receptor substrate-1; Akt; GLUT-4

AEROBIC EXERCISE HAS LONG been advocated as an effective treatment modality for insulin resistance primarily because of its beneficial effects on carbohydrate metabolism and insulin sensitivity (10). These favorable improvements in response to aerobic exercise training are principally the result of qualitative changes within skeletal muscle. An important adaptation to chronic aerobic exercise training is an increase in total skeletal muscle glucose transporter 4 (GLUT-4) protein concentration (49). The increase in GLUT-4 protein concentration is significant because its concentration is directly related to increased rates of insulin-stimulated skeletal muscle glucose transport (5, 15, 48, 49). Chronic aerobic exercise has also been shown to enhance insulin-stimulated insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol-3 kinase (PI3-kinase) activity and glucose uptake in normal human (24) and rodent skeletal muscle (8).

Whereas aerobic exercise has been extensively studied and is well recognized for its beneficial effects on skeletal muscle carbohydrate metabolism, the effect of resistance training has received considerably less attention. A body of literature exists that indicates that resistance training will improve whole body carbohydrate metabolism and insulin action in men and women of different ages (7, 13, 30, 31, 34, 42, 50). It has been suggested that these improvements in whole body carbohydrate metabolism, as a result of resistance training, are primarily due to increases in lean body mass (31). However, hypertrophy may not be necessary for insulin-stimulated carbohydrate metabolism to be improved in normal skeletal muscle. We have demonstrated that chronic resistance training can favorably improve insulin-stimulated skeletal muscle glucose uptake and 3-*O*-[^{3}H]methyl-D-glucose (3-MG) transport in resistance-trained rodents independent of increases in total hindlimb muscle mass (49). Although we attributed the improvements to an increased skeletal muscle GLUT-4 protein concentration, this change alone could not fully account for increased rates of insulin-stimulated glucose transport in the resistance-trained skeletal muscle. Thus it is conceivable that resistance training may have also altered components of the insulin signaling cascade. Therefore, the first aim of this investigation was to more fully evaluate the mechanism by which resistance training enhances insulin-stimulated glucose transport in normal rodent skeletal muscle.

The findings from our laboratory's previous investigation also raised the possibility that resistance training might be capable of directly improving skeletal muscle insulin resistance. A number of investigations have utilized a high-fat-diet rodent model to evaluate skeletal muscle insulin resistance (14, 20, 44). Using this model, our group has reported that a high-fat diet impairs carbohydrate metabolism in rodent skeletal muscle, in part, by reducing total and plasma membrane GLUT-4 protein concentrations, which appears to partially account for decreased rates of insulin-stimulated glucose trans-

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port (40, 48). In addition, our laboratory has recently reported that a high-fat diet impairs insulin-stimulated IRS-1-associated PI3-kinase activity (40). In light of our previous work with a high-fat-fed rodent model, we believed that this model could be useful to determine whether resistance training might favorably improve high-fat diet-induced skeletal muscle insulin resistance. Thus the second aim of this investigation was to evaluate whether chronic resistance training could reverse high-fat diet-induced skeletal muscle insulin resistance and, if so, whether the improvements are accounted for by alterations in components of the insulin signaling cascade and/or the glucose transporter effector system.

METHODS

Experimental design. Thirty-two male Sprague-Dawley rats ~6 wk of age weighing ~240–250 g were obtained from Animal Technologies Limited (Fremont, CA) and randomly assigned to one of two groups: *1*) control (Con, $n = 16$) and *2*) high-fat (HF, $n = 16$). Con animals received a normal diet (no. 112386, Dyets, Bethlehem, PA; 63% carbohydrate, 17% fat, and 20% protein) and high-fat animals consumed a high-fat diet (no. 112387, Dyets; 26% carbohydrate, 59% fat, and 15% protein). The vitamin and mineral content was identical for all animal groups. A similar diet has been shown to induce skeletal muscle insulin resistance in male Sprague-Dawley rats (48). Rats were housed two per cage in a temperature-controlled environment maintained at 21°C with an artificial 12:12-h light-dark cycle. Animals were provided their respective diets and water ad libitum for 9 wk. After the 9-wk dietary period, initial animal groups were further subdivided into control diet, sedentary (Con-Sed, $n = 8$); control diet, resistance trained (Con-RT, $n = 8$); high-fat diet, sedentary (HF-Sed, $n = 8$); and high-fat diet, resistance trained (HF-RT, $n = 8$). Animals remained on their respective diets throughout the training period.

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.

Resistance training. The frequency and intensity of the resistance training were similar to our previous investigation (49). The Con-RT and HF-RT animals performed three sets of 10 repetitions at 75% of one-repetition maximum (1-RM) three times per week for 12 wk on a squat apparatus modeled on a Smith Machine (Fig. 1). We had previously utilized the squat apparatus described by Tamaki et al. (43) but chose to redesign the training apparatus for the present investigation. When using the Tamaki squatter the training appeared to resemble plyometric training. Specifically, animals jumped up from the platform during the concentric phase of the movement and then landed on the platform while still loaded with the weight and on landing attempted to decelerate, which resulted in substantial eccentric loading of the quadriceps. In contrast, when using the redesigned apparatus animals never left contact with the platform, and consequently the movement more closely resembled a traditional squat movement. The modified apparatus consisted of two 45-cm vertical metal rods 0.5 cm in diameter set 31 cm apart on a 15-cm × 20-cm stainless steel metal base securely inserted into a 2.54-cm-thick wood platform. A 33-cm × 2.54-cm wood crossbeam was outfitted with two brass sleeves and placed on the two vertical rods, allowing for uninhibited vertical movement. An aluminum holder, molded to accommodate the rats, was attached to the center of the crossbeam at a 90° angle relative to the base. Animals were strapped into a nylon vest and attached to the aluminum holder with Velcro straps. Animals were placed in a squat position using safety stoppers on each rod to support the load. Two 5-cm metal pegs attached vertically on opposite ends of the crossbeam were used to mount calibrated miniature weight plates. A brief electrical stimulus (10 V, 0.3-s duration) was delivered

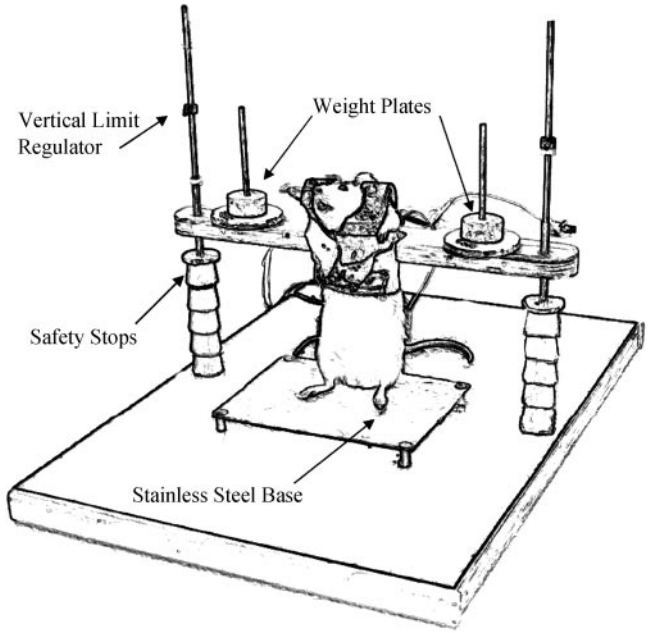

Fig. 1. Resistance-training apparatus.

by manually depressing a switch that allowed current to flow through an electrode attached to the tail of the animals. After each squat, the animals were repositioned on the apparatus such that their legs were beneath the torso. A repetition was initiated by manual electrical stimulation every 15 s and repeated until 10 repetitions were completed. The animals were allowed to rest for 2 min between each set and remained in the apparatus in a standing position during the rest period with the rubber safety stoppers supporting the load. 1-RM was determined for all experimental animals before and at the completion of the training period. During the resistance-training period, 1-RM was determined on a weekly basis for only the Con-RT and HF-RT groups.

Surgical preparation and hindlimb perfusions. After the 12 wk of resistance training and 36–40 h after the last exercise bout, animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (6.5 mg/100 g body wt) and surgically prepared for hindlimb perfusion as described previously by Ruderman et al. (36) and modified by Ivy et al. (18). After the surgical preparation, the left iliac artery was tied off and portions of the red (RG) and white (WG) gastrocnemius, portions of the red (RQ) and white (WQ) quadriceps, gluteus maximus (GM), and hamstring (Ham) were removed from the left leg, freeze clamped in liquid N_2, and stored at −80°C until analysis. IRS-1, Akt, atypical protein kinase C (aPKC)-ζ/λ, and/or GLUT-4 protein concentrations were assessed in these muscles. Rats were perfused as previously detailed (47, 48). Perfusions were performed in the presence of 500 μU/ml of insulin concentration for all experimental groups. Over the first 20-min period, the rate of glucose uptake was assessed in which the perfusate contained 8 mM glucose. Subsequent to the determination of glucose uptake, the hindlimb was washed out with glucose-free perfusate for 1 min in preparation for the measurement of glucose transport. Glucose transport was assessed for an 8-min period in which the perfusate contained an 8 mM concentration of the nonmetabolizable glucose analog 3-MG (32 μCi 3-MG/mmol, PerkinElmer Life Sciences, Boston, MA) and 2 mM 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 (47, 48). Immediately after the transport period, portions of the RG, WG, RQ, WQ, GM, and Ham were excised, blotted on gauze dampened with cold Krebs-Henseleit buffer, and freeze clamped. Muscles were stored at −80° C until 3-MG

transport rates, PI3-kinase activity, Akt phosphorylation, Akt activity, and/or aPKC-ζ/λ activity was assessed. The mass of the hindlimb muscle was determined by adding the mass of the RG, WG, RQ, WQ, GM, and Ham to the mass of remaining muscle that was dissected from the hindlimb (39). The mass of the whole RG, WG, RQ, WQ, GM, and Ham was determined after freeze clamping when the samples were being weighed for 3-MG transport.

Western blotting. Muscles were cut, weighed frozen, and homogenized as previously described (40). The supernatant was extracted, and protein concentration was quantified on the basis of the Bradford method (4) by use of a Benchmark microplate reader (Bio-Rad, Richmond, CA). One hundred microliters of sample lysate were diluted 1:1 with Laemmli sample buffer (28). Seventy micrograms of protein for IRS-1, Akt, and GLUT-4, and 140 μg for aPKC-ζ/λ were subjected to SDS-PAGE run under reducing conditions on a 7.5% (IRS-1, GLUT-4) or 10% resolving gel (Akt, aPKC-ζ/λ) on a Mini-Protean 3 dual-slab cell (Bio-Rad) and transferred to polyvinylidene difluoride membranes by using a semidry transfer unit. The membranes were blocked in 5% nonfat dry milk/Tris-Tween-buffered saline and incubated with either affinity-purified rabbit polyclonal anti-IRS-1 [sc-559, Santa Cruz Biotechnology (SCBT), Santa Cruz, CA], anti-Akt 1/2 (sc-8312, SCBT), anti-PKC-ζ/λ (sc-216, SCBT) or anti-GLUT-4 (donated by Dr. Samuel W. Cushman, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD) followed by an incubation with goat anti-rabbit IgG conjugated to horseradish peroxidase (sc-2004, SCBT). Antibody binding was visualized by enhanced chemiluminescence in accordance to the manufacturer's instructions (West Femto, Pierce Chemical, Rockford, IL). Images were captured by using a charge-coupled device camera in a ChemiDoc system (Bio-Rad) and saved to a Macintosh G4 computer. Bands were quantified with Quantity One analysis software (Bio-Rad). By using the freehand contour tool in the Quantity One analysis software, the density of the bands was determined and expressed as a percentage of a standard sample run on each gel.

Determination of IRS-1-associated PI3-kinase activity. Between 100 and 150 mg of insulin-stimulated muscle tissue were weighed frozen and homogenized as previously described (40). The supernatant was collected and quantified for protein content by the Bradford method (4), and IRS-1-associated PI3-kinase activity was assessed as described elsewhere (40). After a 1-h separation of the phosphoinositides on a thin-layer chromatography plate, the plates were dried and exposed to a storage phosphor screen, the screen was scanned with a phosphor imager (Personal Molecular Imager FX System, Bio-Rad), and the image was imported into a Macintosh G4 computer. Quantification was performed with Quantity One analysis software (Bio-Rad). Kinase activity was calculated as a percentage of an insulin-stimulated muscle standard run on each plate.

Akt phosphorylation. Aliquots of muscle samples that were used for PI3-kinase activity were used to determine Ser473 and Thr308 phosphorylation of Akt. One hundred micrograms of sample protein were subjected to SDS-PAGE run on a 10% resolving gel. The resolved proteins were transferred to polyvinylidene difluoride membranes as described above and blocked in nonfat dry milk/Tris-Tween-buffered saline. The membranes were then incubated with affinity-purified rabbit polyclonal anti-pAkt1 Ser473 (sc-7985, SCBT) or anti-pAkt1/2/3 Thr308 (sc-16646-R, SCBT) followed by incubation with goat anti-rabbit IgG conjugated to horseradish peroxidase. Antibody binding was visualized and quantified as described above. Values were expressed as a percentage of a standard 100-μg insulin-stimulated muscle sample run on each gel.

aPKC-ζ/λ activity. One milligram of insulin-stimulated muscle protein lysate was combined with 2 μg of anti-aPKC-ζ/λ (sc-216, SCBT) and incubated overnight at 4°C. One hundred microliters of a slurry of protein A-Sepharose (Pro-A) beads were then added to each sample and incubated with rotation at 4°C for 1.5 h. After incubation, samples were centrifuged (14,000 rpm, 4°C) for 10 min and the immunocomplex was washed by using the same protocol as described for PI3-kinase activity (40). After the wash protocol, samples were centrifuged (14,000 rpm, 4°C) for 10 min, and the supernatant was discarded. The Pro-A beads were then resuspended in 30 μl of kinase buffer. The kinase reaction was initiated by the addition of 5 μCi [γ-^{32}P]ATP (PerkinElmer Life Sciences), 40 μM/l ATP, and 5 μg myelin basic protein (M8–184, Sigma-Aldrich, St. Louis, MO). The samples were vortexed intermittently while incubating for 12 min at 30°C. The reaction was stopped by the addition of Laemmli buffer and heating for 30 min at 90°C. Samples were briefly centrifuged, and 5 μl of supernatant were subjected to SDS-PAGE run under reducing conditions on a 20% resolving gel. After SDS-PAGE, the gel was wrapped in plastic wrap and exposed to a phosphor screen for 2 h. Images were captured and quantified as described above.

Akt kinase activity. Three hundred micrograms of insulin-stimulated muscle lysate protein were combined with 4 μg of anti-Akt1/2 (SCBT) and incubated overnight at 4°C. One hundred microliters of a slurry of Pro-A beads were then added to each immunoprecipitate and incubated with rotation at 4°C for 1.5 h. After incubation, samples were centrifuged (14,000 rpm, 4°C) for 10 min, and the immunocomplex was washed with the same protocol as described for PI3-kinase activity. After the wash protocol, samples were centrifuged (14,000 rpm, 4°C) for 10 min and the supernatant was completely removed. Ten microliters of assay dilution buffer (Upstate Biotechnology, Lake Placid, NY) was added to the immunocomplex in addition to PKA inhibitor peptide (Upstate Biotechnology) and 10 μCi [γ-^{32}P] ATP (PerkinElmer Life Sciences). Kinase reactions were initiated by the addition of the Crosstide substrate oligopeptide (Upstate Biotechnology), which contains a sequence homologous to glycogen synthase kinase-3, and warmed to 37°C with constant mixing for 10 min. The reaction was terminated by the addition of 80 μl of Tris-tricine sample buffer and heating (95°C) for 5 min. Fifteen microliters were loaded onto a 20% Tris-tricine polyacrylamide gel in duplicate and electrophoresed for 130 min at 100 V using a MiniProtean 3 electrophoresis system (Bio-Rad). After electrophoresis, gels were wrapped in plastic wrap and exposed to a phosphor screen overnight. Images were captured and quantified as described above.

Enzymatic activity. Citrate synthase and hexokinase activity were determined as described elsewhere (49).

Statistical analysis. A one-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 employed to identify statistically significant differences ($P < 0.05$) among the means.

RESULTS

Body, muscle, and epididymal fat mass. The body mass of the Con-Sed (204.8 ± 2.0 g), Con-RT (201.3 ± 1.9 g), HF-Sed (205.4 ± 2.1 g), and HF-RT (200.7 ± 2.5 g) animals was similar at the onset of training. Although the body mass of all groups increased throughout the training period, no significant differences in body mass were observed among Con-Sed (615.6 ± 23.7 g), Con-RT (576.1 ± 20.5 g), HF-Sed (603 ± 42.4 g), and HF-RT (581.8 ± 25.9 g) animals at the end of training. Similarly, at the end of the training period the muscle mass of the right hindlimb was not significantly different among Con-Sed (29.5 ± 0.8 g), Con-RT (27.5 ± 0.8 g), HF-Sed (27.3 ± 1.6 g), and HF-RT (26.6 ± 1.4 g) animals. However, epididymal fat pad mass was greater ($P < 0.05$) in the high-fat-fed animals (HF-Sed: 18.0 ± 2.9 g; HF-RT: 17.9 ± 1.7 g) compared with control diet animals (Con-Sed: 13.9 ± 0.7 g; Con-RT: 13.5 ± 0.7 g).

Resistance training performance. Before the onset of training, the 1-RM was assessed in all groups and no differences existed among Con-Sed (848 ± 147 g), Con-RT (766 ± 74 g),

HF-Sed (790 ± 101 g), and HF-RT (742 ± 87 g). Twelve weeks of resistance training significantly increased the average 1-RM of the Con-RT (1,304 ± 60 g) and HF-RT (1,387 ± 112 g) compared with the Con-Sed (819 ± 70 g) and HF-Sed (864 ± 64 g) animals.

Skeletal muscle glucose uptake and 3-MG transport. The rates of insulin-stimulated skeletal muscle glucose uptake were significantly reduced in the HF-Sed animals compared with Con-Sed animals (Table 1). Skeletal muscle glucose uptake was increased above that of the sedentary animals for both the Con-RT and HF-RT groups.

Rates of insulin-stimulated 3-MG transport were reduced in the RG, RQ, and GM of the HF-Sed animals compared with Con-Sed animals (Table 1). Resistance training reversed the effects of the high-fat diet as evidenced by the improvements in rates of 3-MG transport in the RG and GM of the HF-RT animals. Of interest, resistance training not only reversed the effects of the high-fat diet in the RQ of the HF-RT animals but elevated rates of 3-MG transport to the same extent as that observed in the Con-RT animals. With respect to the Con-RT animals, rates of 3-MG transport were elevated in the RG and RQ compared with Con-Sed animals. Neither the high-fat diet nor resistance training altered 3-MG transport in the WG, WQ, and Ham.

The possibility existed that resistance exercise training might have increased basal rates of glucose metabolism and accounted, in part, for some of the insulin-stimulated training adaptations observed for glucose uptake and transport. To address this concern, we perfused an additional group of Con-Sed and Con-RT animals in the absence of insulin. We found that basal rates of skeletal muscle glucose uptake and 3-MG transport were identical in this subset of control and resistance-trained animals (Table 1), and therefore we did not further analyze this tissue.

IRS-1 protein concentration. Total IRS-1 protein concentration was similar in the muscles evaluated among the experimental groups (Fig. 2*A*).

IRS-1-associated PI3-kinase activity. IRS-1-associated PI3-kinase activity (Fig. 3) was significantly decreased in the RQ of the HF-Sed group compared with Con-Sed ($P < 0.05$). The resistance training significantly elevated insulin-stimulated IRS-1-associated PI3-kinase activity in the RQ of the Con-RT and HF-RT groups compared with Con-Sed and HF-Sed, respectively ($P < 0.05$). In the WQ, no difference in IRS-1-associated PI3-kinase activity was found among the experimental groups.

Akt protein concentration, phosphorylation and activity. No difference in the total protein concentration of Akt 1/2 (Fig. 2*B*) was found in the muscles evaluated among the experimental groups. Insulin-stimulated phosphorylation of Ser473 (Fig. 2*C*) and Thr308 (Fig. 2*D*) residues of Akt were found to be similar in the muscles evaluated among the experimental groups.

The high-fat diet impaired insulin-stimulated Akt activity in the RQ of the HF-Sed group compared with the other groups of animals ($P < 0.05$) (Fig. 4). Twelve weeks of resistance training reversed the effects of a high-fat diet by normalizing Akt activity in the RQ such that no differences existed between the Con-Sed and HF-RT groups, but resistance training in normal skeletal muscle (i.e., Con-RT) did not further increase Akt activity. In the WQ, Akt activity was similar among groups.

aPKC protein concentration and activity. Total aPKC-ζ (Fig. 2*E*) and aPKC-λ (Fig. 2*F*) protein concentration were similar in the RQ and WQ among the experimental groups.

The high-fat diet attenuated insulin-stimulated aPKC-ζ/λ activity in the RQ of the HF-Sed group compared with all other groups ($P < 0.05$) (Fig. 5). Twelve weeks of resistance training significantly elevated aPKC-ζ/λ activity in the RQ of the Con-RT compared with all other experimental groups ($P < 0.05$). Of interest, resistance training reversed the effects of a high-fat diet by normalizing aPKC-ζ/λ activity in the RQ such that no differences existed between the Con-Sed and HF-RT groups ($P < 0.05$). In the WQ, aPKC-ζ/λ activity was similar among groups.

GLUT-4 protein concentration. Total GLUT-4 protein concentration (Fig. 6) in the RG and RQ was significantly decreased in the HF-Sed group compared with Con-Sed ($P < 0.05$). In response to resistance training, total GLUT-4 protein concentration was significantly elevated in the RG, RQ, and WQ of the Con-RT group compared with Con-Sed ($P < 0.05$). Resistance training counteracted the effects of the high-fat diet as evidenced by total GLUT-4 protein concentration being increased in the RQ and normalized in the RG of the HF-RT animals. Neither resistance training nor the high-fat diet affected the GLUT-4 concentration in the WG.

Enzymatic activity. Citrate synthase activity was not different in the skeletal muscle evaluated among the experimental groups (Table 2). Similarly, hexokinase activity (Table 2) was not different among the muscles evaluated, with the exception of the WQ of the HF-RT animals exhibiting a higher activity than that of the WQ from the Con-Sed animals.

Table 1. *Rates of glucose uptake and 3-MG transport*

	Insulin Stimulated				Basal	
	Con-Sed	Con-RT	HF-Sed	HF-RT	Con-Sed	Con-RT
Glucose uptake, $\mu mol \cdot g^{-1} \cdot h^{-1}$	6.78±0.5	9.25±0.6*†	5.04±0.3*	10.90±1.0*†	4.51±0.4	4.27±0.5
3-MG transport $\mu mol \cdot h^{-1} \cdot g^{-1}$						
RG	4.93±0.6	6.50±0.8*†	3.77±0.4*	5.90±0.6*†	3.12±0.3	3.38±0.3
WG	2.80±0.7	3.57±1.3	2.74±0.4	3.19±0.6	1.85±0.1	2.08±0.2
RQ	5.12±0.4	8.03±0.6*†	3.60±0.4*†	7.88±0.5*†	3.55±0.3	3.30±0.3
WQ	2.91±0.5	3.03±0.5	2.74±0.4	2.96±0.3	1.8±0.3	1.2±0.4
GM	3.61±0.4	4.44±0.3†	2.37±0.2*	3.62±0.5†	1.19±0.3	1.18±0.4
Ham	2.88±0.7	2.47±0.4	2.46±0.6	3.57±0.5	0.78±0.4	0.56±0.2

Values are means ± SE. Con-Sed, control diet-sedentary; Con-RT, control diet-resistance trained; HF-Sed, high-fat diet-sedentary; HF-RT, high-fat diet-resistance trained. RG, red gastrocnemius; WG, white gastrocnemius; RQ, red quadriceps; WQ, white quadriceps; GM, gluteus maximus; Ham, hamstring; 3-MG, 3-*O*-[^{3}H]methyl-D-glucose. *Significantly different from Con-Sed ($P < 0.05$). †Significantly different from HF-Sed ($P < 0.05$).

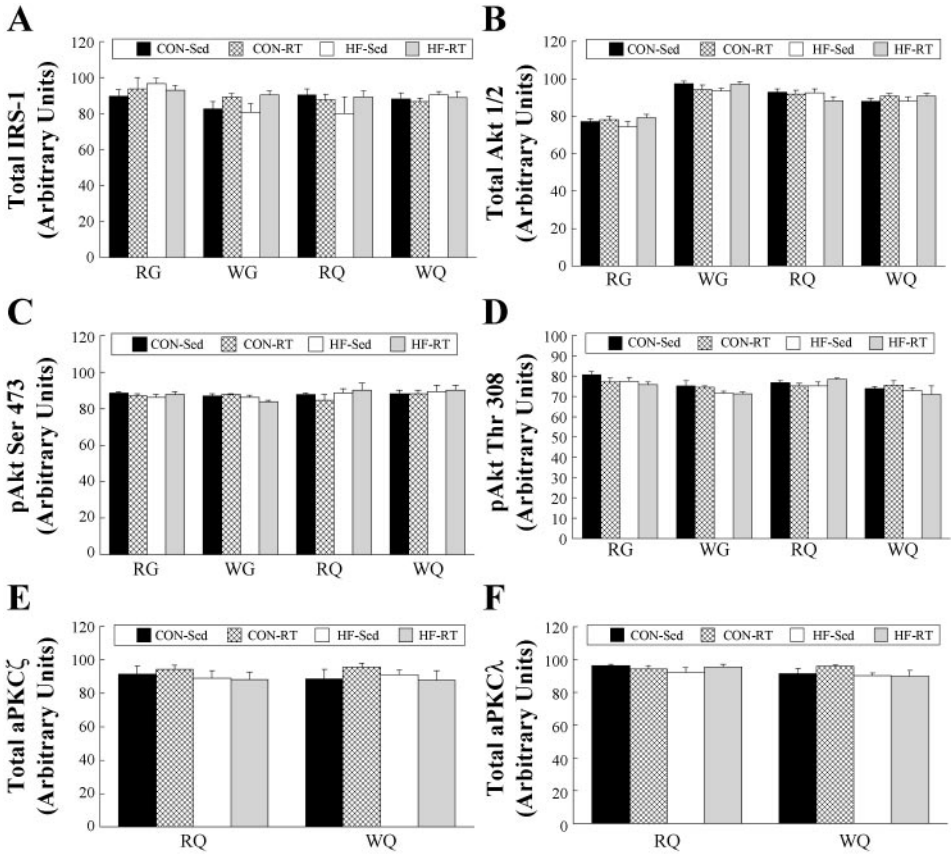

Fig. 2. Total insulin receptor substitute-1 (IRS-1; *A*), Akt 1/2 (*B*), pAkt Ser473 (*C*), pAkt Thr308 (*D*), atypical PKC (aPKC)-ζ (*E*), and aPKC-λ (*F*) protein concentrations. Con-Sed, control, diet sedentary; Con-RT, control diet, resistance trained; HF-Sed, high-fat diet, sedentary; HF-RT, high-fat diet, resistance trained; RG, red gastrocnemius; WG, white gastrocnemius; RQ, red quadriceps; WQ, white quadriceps. No differences existed within muscles among experimental groups.

DISCUSSION

Resistance training significantly improved rates of insulin-stimulated 3-MG transport and skeletal muscle GLUT-4 protein concentration in the Con-RT group compared with Con-Sed animals. We did, however, note that the resistance training resulted in the GLUT-4 protein concentration being differentially affected among the muscle groups evaluated in the present investigation compared with our laboratory's previous study (49). Specifically, in the RG, RQ, and WQ the GLUT-4 protein concentration was elevated to a greater extent, but it was unaltered in the WG. We attribute these differences between our investigations to the redesigned squat apparatus. Although we suggested that the improvements in rates of insulin-stimulated glucose uptake and transport in normal re-

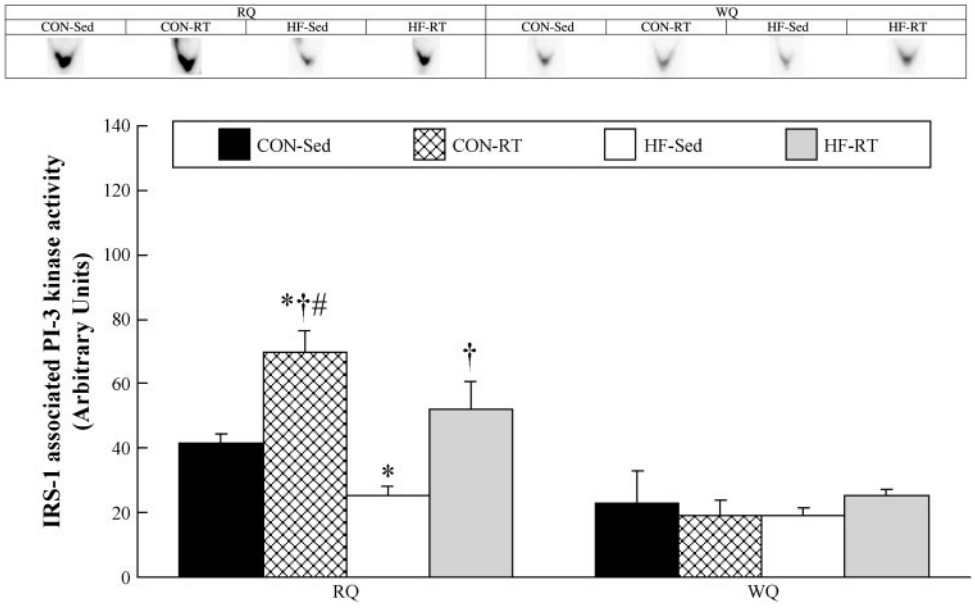

Fig. 3. IRS-1-associated phosphatidylinositol 3 (PI3)-kinase activity, expressed as a percentage of a standard, in skeletal muscles from rats that were divided among 4 groups: Con-Sed, Con-RT, HF-Sed, and HF-RT. *Significantly different from Con-Sed ($P < 0.05$). †Significantly different from HF-Sed ($P < 0.05$). #Significantly different from HF-RT ($P < 0.05$). Values are means ± SE.

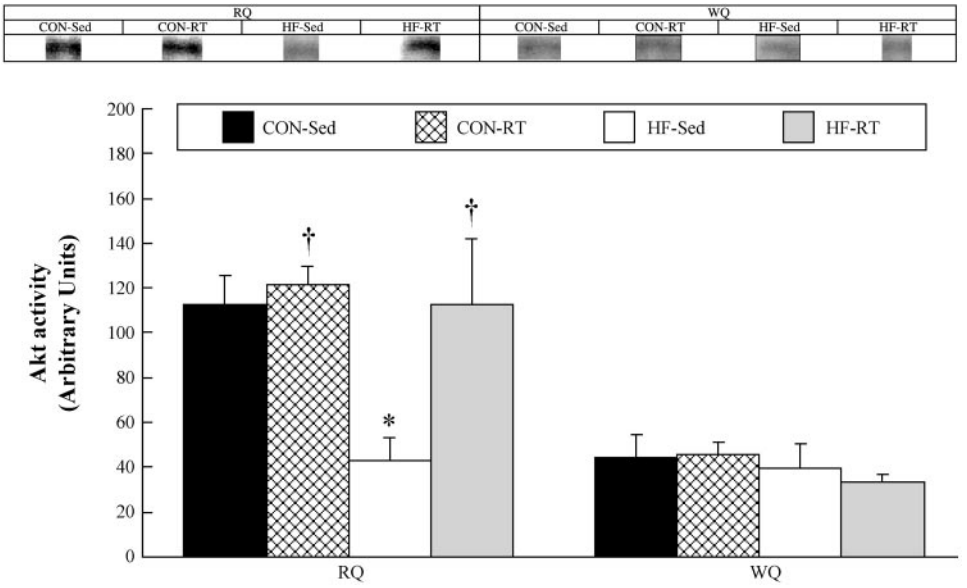

Fig. 4. Akt kinase activity, expressed as a percentage of a standard, in skeletal muscles from rats that were divided among 4 groups: Con-Sed, Con-RT, HF-Sed, and HF-RT. *Significantly different from Con-Sed ($P < 0.05$). †Significantly different from HF-Sed ($P < 0.05$). Values are means ± SE.

sistance-trained skeletal muscle resulted from an increased GLUT-4 protein concentration (49), GLUT-4 protein alone could not fully account for the increased rates of 3-MG transport. In addition, changes in insulin sensitivity are not always associated with an altered skeletal muscle GLUT-4 protein concentration (2, 11, 26). Thus resistance training might also be altering components of the insulin signaling cascade, which was one of the primary questions addressed in the present investigation.

Resistance training appeared to improve insulin-stimulated glucose transport in normal red muscle by increasing IRS-1-associated PI3-kinase activity, aPKC-ζ/λ activity, and total GLUT-4 protein concentration. It is difficult to compare these results to other investigations because we are unaware of any other reports detailing the effects of resistance training on components of the insulin signaling cascade in normal skeletal muscle. Thus we believed that some insight might be gained by comparing and contrasting the effects of aerobic training to that of resistance training. Surprisingly, there is a limited body of literature that has evaluated the effects of aerobic training on components of the insulin signaling cascade in normal rodent skeletal muscle, and these studies have utilized acute and/or short-term rather than chronic aerobic exercise training. Nevertheless, regardless of the mode, duration, and/or frequency of the activity, there appear to be some general training adaptations that occur in the insulin signaling cascade. Consistent with our findings using resistance training, aerobic exercise training increases IRS-1-associated PI3-kinase activity (8) and GLUT-4 protein concentration (35, 49) whereas Akt protein concentration remains unaltered (37). However, skeletal muscle Akt phosphorylation and kinase activity are increased in response to aerobic training (8, 37). This inconsistency may be due in part to differences in the mode and duration of exercise training as well as which Akt isoforms were assessed. To the

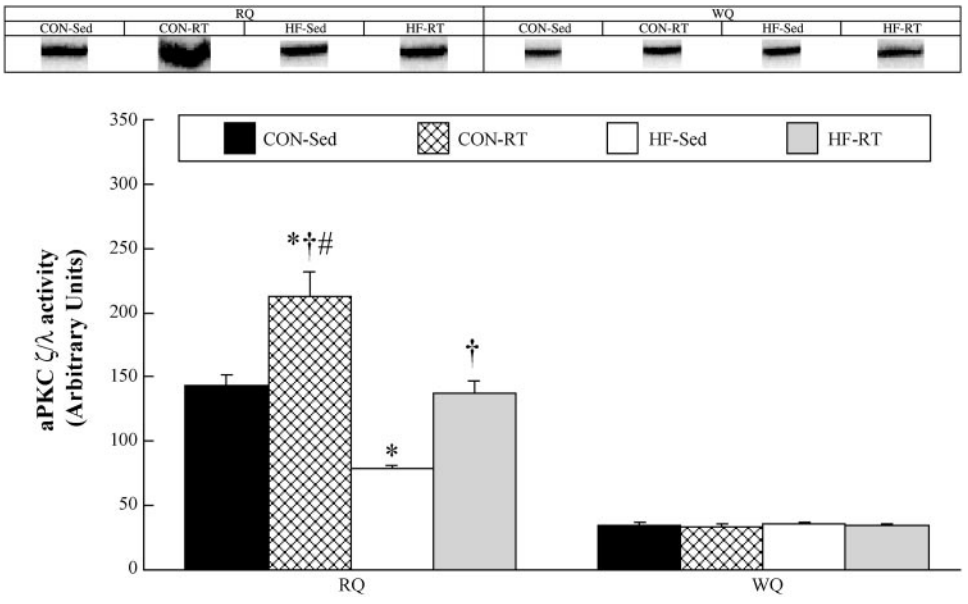

Fig. 5. aPKC-ζ/λ kinase activity, expressed as a percentage of a standard, in skeletal muscles from rats that were divided among 4 groups: Con-Sed, Con-RT, HF-Sed, and HF-RT. *Significantly different from Con-Sed ($P < 0.05$). †Significantly different from HF-Sed ($P < 0.05$). #Significantly different from HF-RT ($P < 0.05$). Values are means ± SE.

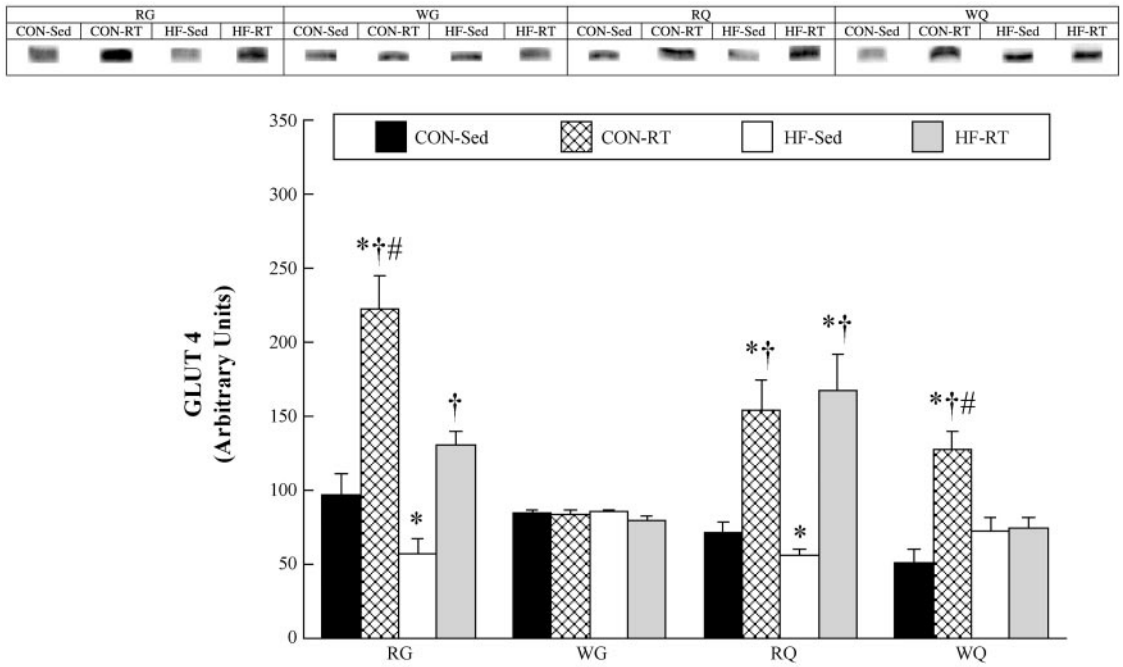

Fig. 6. Total GLUT-4 protein concentration, expressed as a percentage of a standard, in skeletal muscles from rats that were divided among 4 groups: Con-Sed, Con-RT, HF-Sed, and HF-RT. *Significantly different from Con-Sed ($P < 0.05$). †Significantly different from HF-Sed ($P < 0.05$). #Significantly different from HF-RT ($P < 0.05$). Values are means ± SE.

best of our knowledge, aPKC-ζ/λ protein content and activity have yet to be evaluated in aerobically trained normal rodent skeletal muscle.

Rates of insulin-stimulated 3-MG transport have been reported to be related to the total GLUT-4 protein concentration (2, 5, 16, 49). In agreement with these investigations, we found that the high-fat diet suppressed rates of insulin-stimulated 3-MG transport and total GLUT-4 protein concentration in the RG and RQ without any significant effect in the WG. This observation is consistent with previous investigations that have reported that GLUT-4 protein concentration in skeletal muscle of high-fat-fed rodents is reduced (20, 40, 48), and that a high-fat diet decreases 3-MG transport in the RG without any effect in the WG (40, 48).

Although it appears that a decreased GLUT-4 protein concentration can partly explain the reduced rates of insulin-stimulated glucose transport and uptake in high-fat-fed skeletal muscle, other mechanisms may also contribute to impairing skeletal muscle carbohydrate metabolism (29, 33, 40, 44). In a high-fat-fed rodent model it has been proposed that insulin resistance is a consequence of multiple defects in the insulin signaling cascade that ultimately result in the decreased translocation of GLUT-4 to the plasma membrane (14, 44). We found that the high-fat diet did not alter IRS-1 protein concentration, which is consistent with a number of other models of insulin resistance (40, 41, 44). However, insulin-stimulated IRS-1-associated PI3-kinase activity was significantly suppressed in response to the high-fat diet and in agreement with previous investigations that have also reported that IRS-1-associated PI3-kinase activity is decreased in high-fat-fed animals (40, 44). Whereas impaired PI3-kinase activity correlates to decreased glucose transport (22, 25, 29, 38, 41), the mechanism responsible for the high-fat diet reducing insulin-stimulated IRS-1-associated PI3-kinase activity remains to be fully elucidated.

The activation of proteins downstream of PI3-kinase may also be affected in insulin-resistant skeletal muscle. It is known that activation of Akt by PI3-kinase is reliant on phosphorylation of Thr308 and Ser473 residues by PDK-1 (45) and PDK-2 (1), respectively. However, there is some debate concerning the role of Akt in the activation of the glucose transporter effector system in that several investigations have shown phosphorylation of Akt to be impaired in various models of insulin resistance (32, 46), whereas other reports indicate no change in the phosphorylation status of Akt in insulin-resistant tissue (23, 27). In addition, recent studies suggest that activation of aPKC-ζ/λ, also a downstream target of PI 3-kinase, is suppressed in insulin-resistant skeletal muscle (44, 46) and appears to be negatively affected by high-fat feeding (44).

Table 2. *Citrate synthase and hexokinase activity*

	Con-Sed	Con-RT	HF-Sed	HF-RT
Citrate synthase, μmol·g^{-1}·min^{-1}				
RG	44.22±1.1	44.56±4.2	43.19±2.8	42.21±2.8
WG	26.91±1.3	26.34±2.7	27.65±2.3	27.86±2.9
RQ	76.04±6.2	68.10±5.3	71.55±6.1	72.85±5.6
WQ	29.51±5.1	27.21±3.4	30.72±3.1	25.21±2.1
GM	31.24±3.4	29.44±3.4	28.44±2.5	35.74±4.8
Ham	34.76±3.8	36.05±4.7	37.08±5.4	38.26±5.1
Hexokinase, μmol·g^{-1}·min^{-1}				
RG	1.76±0.1	1.56±0.2	1.64±0.1	1.71±0.2
WG	1.53±0.1	1.66±0.1	1.72±0.1	1.64±0.2
RQ	1.63±0.1	1.46±0.1	1.55±0.1	1.48±0.1
WQ	0.96±0.04	1.11±0.1	1.23±0.2	1.42±0.2*
GM	1.46±0.1	1.55±0.1	1.52±0.1	1.62±0.1
Ham	2.88±0.7	2.47±0.4	2.46±0.6	3.57±0.5

Values are means ± SE. *Significantly different from Con-Sed ($P < 0.05$).

We found that not only was Akt 1/2 protein concentration unaffected by the high-fat diet but also that Akt Ser473 and Thr308 phosphorylation were unaltered. Despite normal Akt Ser473 and Thr308 phosphorylation, we show that insulin-stimulated Akt kinase activity was reduced in the skeletal muscle of the HF-Sed animals. Although these data were initially perplexing, on review of the literature we found that this was not the first time that these observations have been made in the high-fat-fed rodent model. Tremblay et al. (44) have also reported that a high-fat diet does not alter Akt-1/2 protein concentration, Akt Ser473 phosphorylation, and Akt Thr308 phosphorylation but does reduce insulin-stimulated Akt activity in rodent skeletal muscle. There is a considerable void in the literature that would justify the provision of a plausible mechanism, and thus we agree with Tremblay et al. that additional work is needed to elucidate the mechanism of the defect in Akt kinase activity. However, several recent studies suggest an isoform-specific defect in Akt activation. Brozinick et al. (6) reported that in insulin-resistant human skeletal muscle Akt-2 and -3 activation is impaired and related to reduced rates of glucose uptake. Using siRNA in 3T3-L1 adipocytes against Akt-1 and Akt-2, Jiang et al. (19) have reported that Akt-2 alone accounts for the majority of insulin-regulated glucose uptake. Thus these data clearly suggest that future investigative routes using the high-fat-fed rodent model should focus on isoform-specific regulation of Akt kinase and mechanisms that impair their insulin-stimulated activation.

We also found the high-fat diet significantly impaired activation of aPKC-ζ/λ activity despite an unchanged aPKC-ζ/λ protein concentration. Again, these findings are in excellent agreement with previous investigations that have reported that a high-fat diet does not alter aPKC-ζ/λ protein concentration but does reduce insulin-stimulated aPKC-ζ/λ activity in rodent skeletal muscle (21, 44). Kanoh et al. (21) reported that defects in activation of aPKC by insulin in high-fat-fed rodent skeletal muscle could not be explained by diminished PDK-1-dependent phosphorylation of Thr 410 in the PKC-ζ activation loop and that defective aPKC activation appears to be due to impaired responsiveness to PI-3,4,5-$(PO_4)_3$ at activation steps distal to PDK-1-dependent loop phosphorylation. Although the exact mechanism by which high-fat feeding reduces insulin-stimulated aPKC-ζ/λ activity is presently unknown, these data certainly are consistent with recent reports (3, 21) that implicate the activation of aPKC-ζ/λ as being potentially important in the regulation of insulin-stimulated skeletal muscle glucose transport.

Resistance training increased rates of insulin-stimulated glucose transport predominantly in the red muscle of the HF-RT animals compared with both the Con-Sed and HF-Sed animals. The improvements in the red muscle of the HF-RT animals appeared to be due to normalization of insulin-stimulated IRS-1-associated PI3-kinase activity, aPKC-ζ/λ activation, Akt activation, and GLUT-4 protein concentration. To the best of our knowledge, this is the first investigation to report that chronic resistance training can favorably alter components of the insulin signaling cascade and GLUT-4 protein concentration in insulin-resistant rodent skeletal muscle. Consequently, it is not possible to directly compare these findings to previous investigations. However, we again suggest that some insight may be gained by comparing and contrasting the effects of aerobic training on insulin resistance in rodent skeletal muscle to that of resistance training.

Much of the information available evaluating aerobic exercise on skeletal muscle insulin resistance has been obtained by using the obese Zucker rat model. In the obese Zucker rat, it is generally agreed that an increased GLUT-4 protein concentration is one training adaptation that enhances skeletal muscle glucose transport (2, 5, 12). Thus it appears that an increased skeletal muscle GLUT-4 protein concentration may be a universal adaptation in various models of insulin resistance that are subjected to chronic exercise training. In contrast, there is some difference of opinion as to whether aerobic exercise training alters components of the insulin signaling cascade. It has been reported in skeletal muscle of exercise-trained obese Zucker rats that insulin receptor tyrosine phosphorylation and IRS-1 protein concentration may (15, 17) or may not (9) be altered. Furthermore, Christ et al. (9) found that IRS-1-associated PI3-kinase activity in the skeletal muscle of obese Zucker rats was unaltered in response to aerobic training. In contrast, we observed that chronic resistance training enhanced skeletal muscle IRS-1-associated PI3-kinase activity. Although it is unclear whether these differences are experimental model or exercise mode specific, it does appear that alterations in PI3-kinase may not always be associated with exercise training-induced improvements in insulin sensitivity. With respect to the effects of exercise training on proteins downstream of PI3-kinase in insulin-resistant rodent skeletal muscle, there have been limited reports indicating that Akt protein concentration (17) and serine phosphorylation (9) are unaltered.

Finally, it is noteworthy that improvements in skeletal muscle glucose metabolism occurred in the absence of significant hindlimb hypertrophy. Although the optimal program design to induce muscle hypertrophy with resistance training in rodents has not been established, we based the sets and repetitions that the animals performed to more closely parallel the general recommendations for resistance training in humans. It is possible, though, that this volume of resistance training exercise and the use of only one exercise (i.e., squat training) may not have been a sufficient stimulus to significantly increase the hindlimb muscle mass in this rodent model. Nevertheless, this does not take away from the fact that resistance training did improve skeletal muscle carbohydrate metabolism but that improvements appear to be fiber type specific.

In summary, our data suggest that improvements in insulin-stimulated glucose transport in response to chronic resistance training in normal skeletal muscle appear to result from enhanced IRS-1-associated PI3-kinase and aPKC-ζ/λ activity, in addition to an increased GLUT-4 protein concentration. A high-fat diet induces skeletal muscle insulin resistance by impairing insulin-stimulated IRS-1-associated PI3-kinase activity and activation of aPKC-ζ/λ and Akt and by decreasing GLUT-4 protein concentration, which appears to contribute to impaired rates of insulin-stimulated glucose uptake and transport. Chronic resistance training appears to reverse the effects of high-fat diet-induced insulin resistance by enhancing insulin-stimulated IRS-1-associated PI3-kinase activity, aPKC-ζ/λ activity, Akt activity, and total GLUT-4 protein concentration. These findings suggest that chronic resistance training can effectively improve carbohydrate metabolism in normal skeletal muscle and reverse high-fat diet-induced skeletal muscle insulin resistance.

GRANTS

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