Exercise reverses high-fat diet-induced impairments on compartmentalization and activation of components of the insulin-signaling cascade in skeletal muscle

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Yaspelkis BB 3rd, Lessard SJ, Reeder DW, Limon JJ, Saito M, Rivas DA, Kvasha I, Hawley JA. Exercise reverses high-fat diet-induced impairments on compartmentalization and activation of components of the insulin-signaling cascade in skeletal muscle. *Am J Physiol Endocrinol Metab* 293: E941–E949, 2007. First published July 10, 2007; doi:10.1152/ajpendo.00230.2007.—The aims of this investigation were 1) to determine whether endurance exercise training could reverse impairments in insulin-stimulated compartmentalization and/or activation of aPKC ζ/λ and Akt2 in skeletal muscle from high-fat-fed rodents and 2) to assess whether the PPAR γ agonist rosiglitazone could reverse impairments in skeletal muscle insulin signaling typically observed after high-fat feeding. Sprague-Dawley rats were placed on chow (NORCON, $n = 16$) or high-fat ($n = 64$) diets for 4 wk. During a subsequent 4-wk experimental period, high-fat-fed rats were allocated ($n = 16$ /group) to either sedentary control (HFC), exercise training (HFX), rosiglitazone treatment (HFRSG), or a combination of both exercise training and rosiglitazone (HFRX). Following the 4-wk experimental period, animals underwent hindlimb perfusions. Insulin-stimulated plasma membrane-associated aPKC ζ and $-\lambda$ protein concentration, aPKC ζ/λ activity, GLUT4 protein concentration, cytosolic Akt2, and aPKC ζ/λ activities were reduced ($P < 0.05$) in HFC compared with NORCON. Cytosolic Akt2, aPKC ζ , and aPKC λ protein concentrations were not affected in HFC compared with NORCON. Exercise training reversed the deleterious effects of the high-fat diet such that insulin-stimulated compartmentalization and activation of components of the insulin-signaling cascade in HFX were normalized to NORCON. High-fat diet-induced impairments to skeletal muscle glucose metabolism were not reversed by rosiglitazone administration, nor did rosiglitazone augment the effect of exercise. Our findings indicate that chronic exercise training, but not rosiglitazone, reverses high-fat diet induced impairments in compartmentalization and activation of components of the insulin-signaling cascade in skeletal muscle.

rosiglitazone; Akt2 and atypical protein kinase C ζ/λ activities; Akt2, atypical protein kinase C ζ , atypical protein kinase C λ , and glucose transporter 4 protein concentrations

INSULIN INCREASES PHOSPHOINOSITIDE 3-KINASE (PI 3-kinase) activity, which in turn increases activation of both Akt and atypical protein kinase C (aPKC) ζ/λ (9, 14, 33). Akt2 activation is involved in regulating insulin-stimulated glucose transport (10, 29) but does not appear to fully account for GLUT4 translocation (13, 36), as dominant negative Akt expression reduces, but does not totally prevent, glucose transporter 4

(GLUT4) translocation (2, 13, 14). These findings suggest that aPKC ζ/λ functions in parallel with Akt to facilitate insulin-stimulated GLUT4 translocation (1, 50).

It is well established that insulin increases Akt2 and aPKC ζ/λ activity. However, it is not known whether the cellular compartmentalization of these proteins is critical for normal physiological function. Insulin stimulation increases plasma membrane Akt2 concentration in rat adipocytes (19) as well as plasma membrane aPKC ζ/λ concentration and activity in myotubes (6) and 3T3-L1 adipocytes (50). Insulin stimulation results in aPKC ζ/λ translocation to the plasma membrane in muscle cell cultures and also increases its association with GLUT4 vesicles (6). We (23) have recently reported that insulin stimulates translocation of aPKC ζ and $-\lambda$, but not Akt2, to plasma membrane fractions prepared from normal rodent skeletal muscle. Furthermore, in that study we observed that high-fat feeding impaired insulin-stimulated skeletal muscle plasma membrane association and activation of aPKC ζ and $-\lambda$ but did not alter plasma membrane Akt2 content or activity. In contrast, total PI 3-kinase, Akt2, and aPKC ζ/λ activities were reduced in the skeletal muscle of the high-fat-fed animals. These findings suggested that not only is the activation of Akt2 and aPKC ζ/λ important, but the cellular location may also be critical for fully activating skeletal muscle glucose transport. In the high-fat-fed rodent model, endurance exercise training has been reported to reverse insulin resistance (34, 38). However, these early studies did not evaluate the effects of exercise training on insulin signaling. Of interest, it has been reported (44–46) that exercise increases the activation and plasma membrane association of aPKC ζ/λ in human skeletal muscle. Thus, the first aim of this investigation was to assess whether chronic aerobic exercise reverses impairments in insulin-stimulated compartmentalization and/or activation of aPKC ζ/λ and Akt2 in high-fat-fed rodent skeletal muscle. We chose to narrow our focus to Akt2 for this investigation, since it has been reported that the activation of Akt2 (10), but not Akt1 (11), is involved in insulin-stimulated glucose transport.

In addition to endurance exercise training, pharmacological intervention has been shown (27) to be effective for improving whole body insulin sensitivity. It has been suggested that peroxisome proliferator-activated receptor agonists, such as rosiglitazone, improve insulin sensitivity in skeletal muscle

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(42) and that this may be due to rosiglitazone increasing AMPK activity in skeletal muscle, which thereby increases glucose uptake (17, 41). This possibility is intriguing in that exercise training enhances AMPK signaling in skeletal muscle as well and is associated with improvements in insulin-stimulated glucose transport (18). Collectively, these observations raise the possibility that rosiglitazone may exert an "exercise mimetic effect" in skeletal muscle. Alternatively, it has been postulated that rosiglitazone may enhance skeletal muscle insulin sensitivity by reducing the accumulation of lipids, which has been referred to as the "lipid steal hypothesis" (59). Nevertheless, the mechanism by which rosiglitazone might potentially modulate components of the insulin-signaling cascade in skeletal muscle is not well understood. Therefore, the second aim of this investigation was to determine whether rosiglitazone affects insulin-stimulated compartmentalization and/or activation of α PKC ζ/λ and Akt2 in high-fat-fed rodent skeletal muscle in a manner similar to chronic aerobic exercise.

METHODS

Experimental design. Eighty male Sprague-Dawley rats (Harlan, San Diego, CA), ~6 wk old, were placed randomly into the following groups: normal diet (NORCON; $n = 16$) or high-fat diet control (HFC; $n = 64$). The NORCON (D12328; Research Diets, New Brunswick, NJ) consisted of 73.1% carbohydrates, 10.5% fat, and 16.4% protein. The HFC (D12330; Research Diets) contained 25.5% carbohydrates, 58% fat, and 16.4% protein. The animals were on their respective diets for 4 wk and allowed to feed ad libitum, which we have previously shown (39, 49, 57) to induce skeletal muscle insulin resistance in male Sprague-Dawley rats. During the subsequent 4-wk experimental period, HFC rats continued to eat the high-fat diet and were randomly allocated to one of the following groups ($n = 16$ /group): HFC, exercise training (HFX), rosiglitazone treatment (HFRSG), or a combination of both exercise training and rosiglitazone treatment (HFRX). Exercise training consisted of treadmill running for 1 h/day, 5 days/wk at 32 m/min on a 15% incline. The speed was gradually increased during the first week of training such that the animals were running at 32 m/min by the 5th day of training and continued to run at this pace for the duration of the exercise training. We (55, 58) have previously shown that when Sprague-Dawley rats are exercised using this speed and grade, red gastrocnemius (RG) oxidative capacity is significantly increased. Rosiglitazone-treated rats received a diet containing 50 ppm rosiglitazone (GlaxoSmithKline, Stevenage, UK), which they consumed ad libitum at an average dose of $2.08 \pm 0.06 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$. The fifth group of rats (NORCON; $n = 16$) remained on the normal chow diet for the duration of the study (8 wk) and acted as a control group. Following the experimental period, animals were fasted for 8–12 h prior to undergoing hindlimb perfusion. Exercise-trained animals undertook their last training bout 36–48 h prior to hindlimb perfusion. We (43) have previously reported serum glucose, insulin, adiponectin, free fatty acids, and skeletal muscle lipid content for these animals.

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.

Hindlimb perfusions. Animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (6.5 mg/100 g body wt) and surgically prepared for hindlimb perfusion as previously described by Ruderman et al. (47) and modified by Ivy et al. (28). Following surgical preparation, cannulas were inserted into the abdominal aorta and vena cava, and the animals were killed via an intracardiac injection of pentobarbital as the hindlimbs were washed out with 30

ml of Krebs-Henseleit buffer (KHB; pH 7.55). Immediately the cannulas were placed in line with a nonrecirculating perfusion system, and the hindlimbs were allowed to stabilize during a 5-min washout period. The perfusate was continuously gassed with a mixture of 95% O_2 -5% CO_2 and warmed to 37°C. Perfusate flow rate was set at 7.5 ml/min during the stabilization and subsequent perfusion, during which rates of glucose transport were determined.

Perfusions were performed in the presence ($n = 8$ /group) or absence ($n = 8$ /group) of 500 $\mu\text{U/ml}$ insulin. The basic perfusate medium consisted of 30% washed time-expired human erythrocytes (Ogden Medical Center, Ogden, UT), KHB, 4% dialyzed bovine serum albumin (Fisher Scientific, Fair Lawn, NJ), and 0.2 mM pyruvate. The hindlimbs were washed out with perfusate containing 1 mM glucose for 5 min in preparation for the measurement of glucose transport. Glucose transport was measured over an 8-min period using an 8 mM concentration of nonmetabolized glucose analog 3-*O*-methylglucose (3-MG; 32 μCi 3- ^3H]MG/mM; PerkinElmer Life Sciences, Boston, MA) and 2 mM mannitol (60 μCi [1- ^{14}C]mannitol/mM; PerkinElmer Life Sciences). Immediately after the transport period, portions of the RG were excised from both hindlimbs, blotted on gauze dampened with cold KHB, freeze-clamped in liquid N_2 , and stored at -80°C for later analysis.

3-MG transport. Rates of insulin-stimulated skeletal muscle 3-MG transport were calculated as previously described (39, 56, 57).

Muscle homogenization for Western blotting. Portions were cut from the RG, weighed frozen, and homogenized in an ice-cold homogenization buffer (1:10 wt/vol) containing 50.0 mM HEPES (pH 7.6), 150 mM NaCl, 20 mM Na pyrophosphate, 20 mM β -glycerophosphate, 10 mM NaF, 2 mM orthovanadate, 2 mM EDTA, 1% IGEPAL, 10% glycerol, 2 mM phenylmethylsulfonylfluoride, 1 mM MgCl_2 , 1 mM CaCl_2 , 10 $\mu\text{g/ml}$ leupeptin, and 10 $\mu\text{g/ml}$ aprotinin. The homogenate was then transferred to a microcentrifuge tube and centrifuged (19,600 g , 4°C) in a refrigerated microcentrifuge (Micro-max RF; International Equipment, Needham Heights, MA) for 15 min. The supernatant was collected, labeled as lysate, and assayed for protein concentration using the Bradford method (5) adapted for use with a Benchmark microplate reader (Bio-Rad, Richmond, CA).

Plasma membrane fractionation. Plasma membrane fractions were prepared as described previously (42). This procedure provides an enriched plasma membrane fraction and a cytosolic fraction that is devoid of plasma membranes (46). Briefly, a portion of the RG was homogenized in $8\times$ (wt/vol) ice-cold buffer containing 20 mM HEPES (pH 7.4), 2 mM EGTA, 50 mM β -glycerophosphate, 1 mM dithiothreitol, 1 mM Na_3VO_4 , 10% glycerol, 3 mM benzamidine, 10 μM leupeptin, 5 μM pepstatin A, and 1 mM phenylmethylsulfonylfluoride. The homogenate was centrifuged at 100,000 g for 30 min at 4°C, and the supernatant was collected as the cytosolic fraction. The pellet was resuspended by agitation in $4\times$ (wt/vol) ice-cold homogenization buffer, to which 1% Triton X was added. The resuspended pellet was then centrifuged at 15,000 g for 10 min at 4°C. The supernatant, representing the plasma membrane fraction, was collected.

Western blotting. Cytosolic [100 μg of protein for insulin receptor substrate-1 (IRS-1), α PKC ζ , α PKC λ , Akt2, and GLUT4] and plasma membrane samples from the RG (100 μg of protein for α PKC ζ , Akt2, and GLUT4) were added to Laemmli buffer (40). Sample proteins were subjected to SDS-PAGE run under reducing conditions on a 10 or 7.5% (IRS-1) resolving gel in a MiniProtean 3 dual-slab cell (Bio-Rad). Resolved proteins were transferred to polyvinylidene difluoride membranes using a semidry transfer unit (10 V for 55 min). Membranes were then blocked in 5% nonfat milk-Tris-Tween-buffered saline and incubated in anti-Akt2 [cat. no. 07-372; Upstate Biotechnology (UBT), Charlottesville, VA], anti- α PKC ζ/λ [sc-216; Santa Cruz Biotechnology (SCBT), Santa Cruz, CA], anti-IRS-1 (cat. no. 06-248; UBT), or GLUT4 (donated by Dr. Samuel W. Cushman, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD) followed by incubation with goat anti-rabbit IgG-

conjugated horseradish peroxidase (cat. no. 12-348; UBT). Antibody binding was visualized by enhanced chemiluminescence in accordance with the manufacturer's instructions (UBT). Images were captured using a ChemiDoc system (Bio-Rad) equipped with a charge-coupled device camera and saved to a Macintosh G4 computer. Protein bands were quantified as a percentage of a muscle sample standard run on each gel using Quantity One analysis software (Bio-Rad).

Akt2 kinase activity. Two hundred fifty micrograms of either RG cytosolic or plasma membrane protein were combined with 4 μ g of anti-Akt2 (UBT) and incubated overnight at 4°C. One hundred microliters of a slurry containing protein A-Sepharose (Pro-A) beads was added to each immunoprecipitate and incubated with rotation at 4°C for 1.5 hours. After incubation the samples were centrifuged (18,300 g, 4°C) for 10 min, and the immunocomplex was washed with the same protocol as described for PI 3-kinase activity (49). After the wash protocol, the samples were centrifuged (18,300 g, 4°C) for 10 min, and the supernatant was removed. Ten microliters of assay dilution buffer (cat. no. 20-108; UBT) was added to the immunocomplex in addition to PKA inhibitor peptide (cat. no. 12-151; UBT) and 10 μ Ci [γ -³²P]ATP (PerkinElmer Life Sciences). Kinase reactions were initiated by addition of the Crosstide substrate oligopeptide (cat. no. 12-331; UBT) and warmed to 37°C, with constant mixing for 10 min. Reactions were halted by addition of Laemmli buffer (1:1). For analysis, 15 μ l of the sample/Laemmli buffer was loaded onto a 20% Tris-tricine polyacrylamide gel in duplicate and electrophoresed at 100 V for 130 min using a MiniProtean 3 electrophoresis system (Bio-Rad). After electrophoresis, gels were wrapped in plastic wrap and exposed to a phosphor screen for 8 h. Images were captured and quantified as described above.

aPKC ζ / λ activity. Five hundred micrograms of either RG cytosolic or plasma membrane proteins was added to 4 μ g of anti-aPKC ζ / λ (sc-216; SCBT) and incubated overnight at 4°C. One hundred microliters of a slurry containing Pro-A beads was combined with each of the samples and incubated with rotation at 4°C for 1.5 h. After incubation the samples were centrifuged (18,300 g, 4°C) for 10 min, and the immunocomplex was washed by using the same protocol as described for PI 3-kinase activity (49). After the wash protocol, samples were centrifuged (18,300 g, 4°C) for 10 min, and the supernatant was discarded. The Pro-A beads were then resuspended in 75 μ l of kinase buffer. The kinase reaction was started by combining 5 μ Ci [γ -³²P]ATP (PerkinElmer Life Sciences), 40 μ M ATP, and 5 μ l of myelin basic protein (M8-184; Sigma-Aldrich, St. Louis, MO). The samples were vortexed periodically while incubating for 12 min at 37°C. Reactions were stopped by addition of Laemmli buffer (1:1). For analysis, 15 μ l of the sample/Laemmli buffer was subjected to SDS-PAGE run under reducing conditions on a 20% Tris-tricine resolving gel. After electrophoresis the gels were wrapped in plastic wrap and exposed to a phosphor screen for 8 h. Images were captured and quantified as described above.

IRS-1 tyrosine and serine phosphorylation. Sixty microliters of a Pro-A slurry that had been coated with an anti-IRS-1 antibody (cat. no. 06-248; UBT) were added to 500 μ g of RG lysate protein and incubated with rotation at 4°C for 2 h. After incubation the samples were centrifuged (18,300 g, 4°C) for 10 min, and the immunocomplex was washed. The Pro-A beads were resuspended in 30 μ l of Laemmli

buffer, subjected to SDS-PAGE on a 7.5% resolving gel, and transferred to polyvinylidene difluoride membranes as described above. Membranes were then subjected to Western blotting, and the proteins were visualized and quantified as described above using either anti-phosphotyrosine (cat. no. 02-247; UBT) or anti-phospho IRS-1 Ser³⁰⁷ (cat. no. 02-247; UBT) as the primary antibody.

Statistical analysis. A one-way analysis of variance was used on all variables to determine whether significant differences exist between groups. When a significant *F* ratio was obtained, a Tukey HSD post hoc test was used to identify statistically significant differences (*P* < 0.05) among the means. Statistical analyses were performed using JMP software (SAS Institute, Cary, NC), and all values were expressed as means $\pm$ SE.

RESULTS

Body and epididymal fat pad mass. Body and epididymal fat pad mass (Table 1) of the HFC and HFRSG animals were heavier (*P* < 0.05) compared with the body and epididymal fat pad mass of the NORCON, HFX, and HFRX animals.

Glucose transport. Glucose transport data were initially published in Lessard et al. (43). Briefly, basal rates of 3-MG transport were similar among groups. Rates of insulin-stimulated 3-MG transport in HFC, HFRSG, and HFRX were less than NORCON, but 3-MG transport rates were not different between NORCON and HFX. Rates of insulin-stimulated 3-MG transport in the HFX were greater than HFC and HFRSG.

IRS-1 protein concentration and phosphorylation. Cytosolic IRS-1 protein concentration in the HFRSG animals was reduced compared with all other groups (*P* < 0.05; Fig. 1A). In the HFC animals IRS-1 tyrosine phosphorylation was reduced and Ser³⁰⁷ phosphorylation was increased compared with the other animals (*P* < 0.05; Fig. 1B). When Ser³⁰⁷ phosphorylation was expressed relative to IRS-1 protein concentration it was found that serine phosphorylation was increased (*P* < 0.05) in both the HFC and HFRSG animals compared with the NORCON, HFX, and HFRX animals (Fig. 1C).

Akt2 protein concentration. Cytosolic Akt2 protein concentration was not different in the absence or presence of insulin among groups (Fig. 2A). In addition, plasma membrane Akt2 protein concentration was not different among groups in either the absence or presence of insulin (Fig. 2B).

aPKC ζ and - λ protein concentration. Cytosolic aPKC ζ protein concentration was similar among groups in the absence or presence of insulin (Fig. 3A). Similarly, cytosolic PKC λ protein concentration was not different among groups in the absence or presence of insulin, the exception being HFRX, which was greater than that of the NORCON group under basal conditions (*P* < 0.05; Fig. 4A).

In the absence of insulin, plasma membrane-associated aPKC ζ protein concentration of the HFC animals was lower

Table 1. Body mass and fat pad mass lipids were removed from this table

	NORCON	HFC	HFX	HFRSG	HFRX
Body mass, g	436 $\pm$ 6	464 $\pm$ 8*	417 $\pm$ 5#	470 $\pm$ 8*	404 $\pm$ 7#
Epididymal fat pad mass, g	6.2 $\pm$ 0.3	10.7 $\pm$ 0.6*	6.8 $\pm$ 0.4#	10.3 $\pm$ 0.5*	6.6 $\pm$ 0.3#

Values are means $\pm$ SE. NORCON, normal diet, control animals; HFC, high-fat diet, control animals; HFX, high-fat diet, exercise-trained animals; HFRSG, high-fat diet, rosiglitazone-treated animals; HFRX, high-fat diet, rosiglitazone-treated, exercise-trained animals. *Significantly different from NORCON (*P* < 0.05); #significantly different from HFC (*P* < 0.05).

Fig. 1. Insulin receptor substrate-1 (IRS-1) protein concentration (A), IRS-1 tyrosine (pY; B) and Ser³⁰⁷ (pSer) phosphorylation, and ratio of pSer to IRS-1 protein concentration (C) in insulin-stimulated skeletal muscle obtained from normal diet, control (NORCON); high-fat diet, control (HFC); high-fat diet, exercise-trained (HFX); high-fat diet, rosiglitazone-treated (HFRSG); and high-fat diet, rosiglitazone-treated, exercise-trained (HFRX) animals. *Significantly different from NORCON ($P < 0.05$); †significantly different from HFX ($P < 0.05$); #significantly different from HFC ($P < 0.05$); ¥significantly different from HFRX ($P < 0.05$). Values are means $\pm$ SE.

Fig. 2. Cytosolic Akt 2 protein concentration (A) and plasma membrane Akt2 protein concentration (B) obtained from NORCON, HFC, HFX, HFRSG, and HFRX animals. Values are means $\pm$ SE.

($P < 0.05$) compared with NORCON, HFRSG, and HFRX animals (Fig. 3B). Similarly, in the absence of insulin, plasma membrane-associated aPKC λ protein concentration of the HFC animals was lower compared with HFX, HFRSG, and HFRX animals ($P < 0.05$; Fig. 4B). In the presence of insulin, plasma membrane-associated aPKC ζ and $-\lambda$ were increased ($P < 0.05$) above basal levels in all groups. However, insulin-stimulated plasma membrane-associated aPKC ζ protein concentration was reduced in HFC compared with NORCON, HFX, HFRSG, and HFRX ($P < 0.05$; Fig. 3B). Insulin-stimulated plasma membrane-associated aPKC ζ protein concentration in HFRX was greater ($P < 0.05$) compared with NORCON and HFX. Insulin-stimulated plasma membrane-associated aPKC λ protein concentration was reduced in HFC compared with NORCON and HFRX ($P < 0.05$; Fig. 4B).

Akt2 activity. In the absence of insulin, cytosolic Akt2 activity was not different among groups (Fig. 5A). In the presence of insulin, cytosolic Akt 2 activity was increased above basal levels ($P < 0.05$). However, cytosolic insulin-stimulated Akt2 activity of the HFC, HFRSG, and HFRX groups was less than ($P < 0.05$) that of NORCON and HFX.

Fig. 3. Cytosolic atypical protein kinase C ζ (aPKC ζ) protein concentration and (A) plasma membrane aPKC ζ protein concentration (B) from NORCON, HFC, HFX, HFRSG, and HFRX animals. *Significantly different from NORCON ($P < 0.05$); †significantly different from HFC ($P < 0.05$); #significantly different from HFX ($P < 0.05$); §significantly different from group basal condition. Values are means $\pm$ SE.

Although the high-fat diet and insulin affected cytosolic Akt2 activity, plasma membrane Akt2 activity (Fig. 5B) was not different among the experimental groups.

aPKC ζ /λ activity. Cytosolic aPKC ζ /λ activity under basal conditions was similar among NORCON, HFC, and HFX (Fig. 5C). Basal cytosolic aPKC ζ /λ activity in the HFRSG and HFRX groups was elevated ($P < 0.05$) above the NORCON and HFC groups. Insulin stimulation increased cytosolic aPKC ζ /λ kinase activity above basal levels in all groups ($P < 0.05$; Fig. 5C). Insulin-stimulated cytosolic aPKC ζ /λ kinase activity in HFC was lower ($P < 0.05$) compared with NORCON, HFX, and HFRX. Insulin-stimulated cytosolic aPKC ζ /λ kinase activity also differed ($P < 0.05$) between HFRSG and HFRX.

In the absence of insulin, no differences existed in aPKC ζ /λ activity among groups, and insulin increased plasma membrane aPKC ζ /λ activity above basal levels (Fig. 5D). Of particular interest, insulin-stimulated plasma membrane aPKC ζ /λ activity was lower ($P < 0.05$) in HFC, HFRSG, and HFRX compared with both NORCON and HFX (Fig. 5D).

GLUT4 protein concentration. Cytosolic GLUT4 protein concentration was similar among groups in the absence and presence of insulin (Fig. 6A). In the absence of insulin, plasma membrane GLUT4 protein concentration was similar among groups (Fig. 6B). Insulin increased the plasma membrane GLUT4 protein concentration above basal levels for all groups (Fig. 6B). However, insulin-stimulated plasma membrane GLUT4 protein concentration was lower in HFC compared with NORCON, HFX, and HFRX ($P < 0.05$; Fig. 6B). Additionally, insulin-stimulated plasma membrane GLUT4 protein concentration in HFRSG was lower compared with HFX.

DISCUSSION

Insulin-stimulated carbohydrate metabolism was significantly impaired in the skeletal muscle from the high-fat-fed animals, as evidenced by decreased rates of 3-MG transport (43) and plasma membrane GLUT4 protein concentration compared with the normal-diet animals. These observations are in agreement with a number of previous reports that have also shown a high-fat diet to decrease insulin-stimulated glucose

Fig. 4. Cytosolic aPKCλ protein concentration (A) and plasma membrane aPKCλ protein concentration (B) obtained from NORCON, HFC, HFX, HFRSG, and HFRX animals. *Significantly different from NORCON ($P < 0.05$); #significantly different from HFC ($P < 0.05$); §significantly different from group basal condition. Values are means $\pm$ SE.

Fig. 5. Cytosolic Akt2 activity (A), plasma membrane Akt2 activity (B), cytosolic aPKC ζ/λ activity (C), and plasma membrane aPKC ζ/λ activity (D) obtained from NORCON, HFC, HFX, HFRSG, and HFRX animals. *Significantly different from NORCON ($P < 0.05$); †significantly different from HFX ($P < 0.05$); #significantly different from HFC ($P < 0.05$); ¥significantly different from HFRX ($P < 0.05$); §significantly different from group basal condition. Values are means $\pm$ SE.

transport (4, 8, 20, 21, 37, 39, 49, 54, 56), PI-3 kinase activity (39, 49, 52, 57), and plasma membrane GLUT4 protein concentration (21, 49, 52, 56, 57) in rodent skeletal muscle. Additionally, cytosolic insulin-stimulated Akt2 and aPKC ζ/λ activities were reduced in the high-fat-fed animals, although cytosolic skeletal muscle Akt2, aPKC ζ , and aPKC λ protein concentrations were unaltered. Moreover, these observations are consistent with our recent report (23) and those of other investigators (30, 39, 52) who have also shown Akt2 and aPKC ζ/λ activities to be reduced in the absence of alterations in total protein concentration and phosphorylation.

With regard to the plasma membrane protein concentration and activation of Akt2, we did not find that insulin increased either plasma membrane-associated Akt2 protein concentration or activity above basal levels. In contrast, plasma membrane protein concentration and activation of aPKC ζ and λ protein in the high-fat-fed skeletal muscle were increased above basal levels in response to insulin, but the insulin-stimulated increase in plasma membrane aPKC ζ and λ and aPKC ζ/λ activities in the high-fat-fed skeletal muscle was less than that of the normal-diet animals. These findings are consistent with our previous observations (23).

Aerobic exercise is well recognized for its beneficial effects on skeletal muscle glucose metabolism. Much of the work that has evaluated aerobic exercise on skeletal muscle insulin resistance in a rodent model has been conducted using the obese Zucker rat. In these studies it has been found that aerobic training enhances skeletal muscle GLUT4 protein concentration (3, 7, 15), may (22, 24) or may not (12) alter IRS-1 protein concentration and IRS-1 tyrosine phosphorylation, and does not affect PI-3 kinase activity (12), Akt protein concentration (24), or Akt serine phosphorylation (12). In the high-fat-fed rodent model, chronic aerobic exercise has been reported to

reverse insulin resistance (34, 38), but these studies did not evaluate the effects of aerobic exercise on insulin signaling. In agreement with these reports (34, 38) we observed that chronic aerobic exercise improved skeletal muscle carbohydrate metabolism, as evidenced by insulin-stimulated rates of 3-MG transport being similar in high-fat-fed and normal-diet animals (43). This improvement was not due to training increasing expression of components of the insulin-signaling cascade but rather resulted from insulin being able to more effectively activate components of the insulin-signaling cascade. Specifically, insulin-stimulated activation of cytosolic Akt2, plasma membrane-associated PKC ζ/λ , and cytosolic PKC ζ/λ were normalized in the skeletal muscle of high-fat-fed animals. Of interest, although exercise training did not alter cellular compartmentalization of Akt2, we did find plasma membrane associated aPKC ζ and λ to be increased.

With respect to aPKC ζ/λ , our observations are consistent with several recent reports in human skeletal muscle that show that exercise training increases insulin-stimulated plasma membrane association and activation of aPKC ζ/λ (44–46). Why insulin increases plasma membrane aPKC ζ and λ protein concentration and aPKC ζ/λ activity is not fully understood, but aPKC ζ/λ directly interacts with GLUT4-containing vesicles (6, 31, 50), and Hodgkinson et al. (26) have recently reported that aPKC ζ regulates munc18 (a protein of the GLUT4 vesicular trafficking machinery), which collectively suggests that aPKC ζ/λ assists in GLUT4 translocation. This seems plausible in that insulin-stimulated plasma membrane GLUT4 protein concentration is increased in the high-fat-fed exercise-trained animals. However, this does not minimize the fact that activation of Akt2 is clearly necessary in order for glucose transport to occur in skeletal muscle (10). Rather, it appears that both Akt2 and aPKC ζ/λ are required for glucose

Fig. 6. Cytosolic glucose transporter 4 (GLUT4) protein concentration (A) and plasma membrane GLUT4 protein concentration (B) obtained from NORCON, HFC, HFX, HFRSG, and HFRX animals. *Significantly different from NORCON ($P < 0.05$); †significantly different from HFX ($P < 0.05$); #significantly different from HFC ($P < 0.05$); §significantly different from group basal condition. Values are means $\pm$ SE.

transport (16) and that appropriate cellular compartmentalization is also essential for normal physiological function. Additionally, we (43) have noted that the exercise-induced improvements on insulin-stimulated rates of glucose transport may be related to upregulation of AMPK α 1 activity and decreased AS160 expression.

Although we show downstream components of the insulin-signaling cascade to be negatively affected by high-fat feeding and improved by exercise training, the possibility that these effects are regulated at a shared upstream location in the insulin-signaling cascade seemed plausible. It has been reported that increased IRS-1 serine phosphorylation prevents the insulin-signaling cascade from becoming fully activated (25, 35, 53, 60) and is related to increased diacylglycerol (DAG) accumulation in the skeletal muscle (35, 48). We (43) have previously reported DAG content is elevated in the skeletal muscle of the high-fat-fed animals compared with normal-diet animals and that exercise training reduced DAG content. In the present investigation we show that IRS-1 serine phosphorylation is elevated and that insulin-stimulated IRS-1

tyrosine phosphorylation is reduced in the skeletal muscle of the high-fat-fed animals compared with normal-diet animals. Of interest, we noted that, when high-fat-fed animals were subjected to chronic aerobic exercise, IRS-1 serine phosphorylation was reduced and insulin-stimulated IRS-1 tyrosine phosphorylation increased. We (43) have demonstrated that high-fat feeding increases DAG accumulation due to increased rates of palmitate uptake without a concomitant increase in fatty oxidation. In contrast, exercise training increased palmitate oxidation without increasing palmitate uptake, which prevented DAG accumulation (43). Taken collectively, these data suggest that high-fat feeding initiates impairments on the insulin-signaling cascade in skeletal muscle at IRS-1 as a result of DAG accumulation activating serine kinases, which in turn negatively affects the activation and compartmentalization of the downstream components of the insulin-signaling cascade. However, if high-fat diet-induced DAG accumulation can be reversed, such as which occurs in response to chronic exercise training, then IRS-1 serine phosphorylation is reduced, allowing for appropriate activation of the insulin-signaling cascade.

It has been reported (24) that the PPAR γ agonist troglitazone and exercise training have additive effects on whole body insulin sensitivity in obese Zucker rats, but whether these effects are attributable to improvements in skeletal muscle insulin sensitivity has not been fully elucidated. In contrast to one of our original hypotheses, rosiglitazone did not reverse high-fat diet-induced impairments on insulin-stimulated rates of 3-MG transport in skeletal muscle. This lack of effect of rosiglitazone was further confirmed by our findings that high-fat diet-induced impairments in insulin-stimulated activation and compartmentalization of components of the insulin-signaling cascade were not reversed by rosiglitazone treatment. These findings are not without precedent. Karlsson et al. (32) have previously reported that rosiglitazone does not enhance insulin signaling in type 2 diabetic human skeletal muscle. On the other hand, Todd et al. (51) have reported that rosiglitazone improves insulin-stimulated activation of select components of the insulin-signaling cascade in high-fat-fed rodents. Differences in results between these studies are hard to reconcile but may be related to the concurrent provision of rosiglitazone during a 3-wk period of high-fat feeding (51), whereas in the present investigation rats were initially provided a high-fat diet for 4 wk and then were treated with rosiglitazone in concert with the high-fat diet for an additional 4 wk. It is also possible that the lack of effects of rosiglitazone on glucose transport and insulin-signaling proteins might be a result of the animals consuming a slightly lower dose of rosiglitazone in the present investigation ($\sim 2.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) compared with our previous investigation ($\sim 3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) (42). Of note, we found that rosiglitazone did not reverse the high-fat diet-induced serine phosphorylation of IRS-1 when expressed relative to IRS-1 protein concentration, which could account for the lack of improvement in rosiglitazone-treated animals. In addition, we found a somewhat paradoxical effect of rosiglitazone in that insulin-stimulated plasma membrane GLUT4 protein concentration was similar in NORCON and HFRX animals, but rates of 3-MG transport were not increased in the HFRX. It is likely that 3-MG transport was not increased due to plasma membrane PKC ζ/λ activity being unaffected in the HFRX animals. We interpret this as exercise training increases plasma membrane-associated GLUT4 protein concentration,

possibly due to increased AMPK α 1 activity (43). The key disconnect in the plasma membrane PKC ζ / λ protein concentration and activity is noted only in the rosiglitazone-treated animals. This disconnect may result from a change in lipid composition of the plasma membrane. Activation of PKCs is a two-step process comprising the initial signaling event(s) causing plasma membrane translocation, followed by activation by the lipid product PIP $_3$ (16). Why PKC ζ / λ activity was not altered in the rosiglitazone-treated animals may be due to a change in lipid composition of the plasma membrane that has been observed previously (42). However, this is not to suggest that rosiglitazone is incapable of improving insulin sensitivity in other insulin-responsive tissues, as we (43) have recently reported that rosiglitazone does enhance insulin sensitivity in adipose and liver obtained from high-fat-fed rodents.

In conclusion, we provide novel evidence that high-fat feeding does not alter cytosolic Akt2 or aPKC ζ and λ protein concentrations compared with consumption of a normal diet. Rather, insulin-stimulated cytosolic Akt2 and aPKC ζ / λ activities, insulin-stimulated plasma membrane-associated aPKC ζ and λ protein concentrations, aPKC ζ / λ activity, and GLUT4 protein concentration are reduced in the high-fat-fed animals. Of particular note, aerobic exercise training reversed the effects of the high-fat diet such that insulin-stimulated compartmentalization and activation of components of the insulin-signaling cascade were similar to that of normal-diet control animals. Rosiglitazone did not reverse high-fat diet-induced impairments on glucose metabolism in skeletal muscle, did not augment the effect of exercise, and in some instances even inhibited the positive effects of exercise training. These findings suggest that chronic exercise training, but not rosiglitazone, can reverse high fat-diet-induced impairments in compartmentalization and activation of components of the insulin-signaling cascade in skeletal muscle.

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