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Effects of a brief high-fat diet and acute exercise on the mTORC1 and IKK/NF- κ B pathways in rat skeletal muscle

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Abstract

One exercise session can improve subsequent insulin-stimulated glucose uptake by skeletal muscle in healthy and insulin-resistant individuals. Our first aim was to determine whether a brief (2 weeks) high-fat diet (HFD) that caused muscle insulin resistance would activate the mammalian target of rapamycin complex 1 (mTORC1) and/or inhibitor of κ B kinase/nuclear factor κ B (IKK/NF- κ B) pathways, which are potentially linked to induction of insulin resistance. Our second aim was to determine whether acute exercise that improved insulin-stimulated glucose uptake by muscles would attenuate activation of these pathways. We compared HFD-fed rats with rats fed a low-fat diet (LFD). Some animals from each diet group were sedentary and others were studied 3 h postexercise, when insulin-stimulated glucose uptake was increased. The results did not provide evidence that brief HFD activated either the mTORC1 (including phosphorylation of mTOR^{Ser2448}, TSC2^{Ser939}, p70S6K^{Thr412}, and RPS6^{Ser235/236}) or the IKK/NF- κ B (including abundance of I κ B α or phosphorylation of NF- κ B^{Ser536}, IKK α / β ^{Ser177/181}, and I κ B^{Ser32}) pathway in insulin-resistant muscles. Exercise did not oppose the activation of either pathway, as evidenced by no attenuation of phosphorylation of key proteins in the IKK/NF- κ B pathway (NF- κ B^{Ser536}, IKK α / β ^{Ser177/181}, and I κ B^{Ser32}), unaltered I κ B α abundance, and no attenuation of phosphorylation of key proteins in the mTORC1 pathway (mTOR^{Ser2448}, TSC2^{Ser939}, and RPS6^{Ser235/236}). Instead, exercise induced greater phosphorylation of 2 proteins of the mTORC1

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pathway (PRAS40^{Thr246} and p70S6K^{Thr412}) in insulin-stimulated muscles, regardless of diet. Insulin resistance induced by a brief HFD was not attributable to greater activation of the mTORC1 or the IKK/NF- κ B pathway in muscle, and exercise-induced improvement in insulin sensitivity was not attributable to attenuated activation of these pathways in muscle.

Abstract

Une séance d'exercice peut améliorer subséquemment la captation du glucose médiée par l'insuline dans le muscle squelettique de personnes en bonne santé et insulino-résistantes. Cette étude se propose premièrement de déterminer si un bref (2 semaines) régime alimentaire riche en gras (HFD), cause de l'insulino-résistance musculaire, active les voies mTORC1 et/ou IKK/NF- κ B possiblement associées à l'installation de l'insulino-résistance. Deuxièmement, on veut vérifier si une séance d'exercice qui accroît la captation musculaire du glucose médiée par l'insuline diminue l'activation de ces voies. On compare des rats soumis à un HFD à des rats recevant un régime faible en gras (LFD). Quelques animaux de chaque groupe soumis à un régime sont sédentaires et les autres sont examinés 3 heures après la séance d'exercice au moment où il y a une augmentation de la captation du glucose médiée par l'insuline. Les résultats ne prouvent pas qu'un bref HFD active soit mTORC1 (incluant la phosphorylation des voies mTOR^{Ser2448}, TSC2^{Ser939}, p70S6K^{Thr412} et RPS6^{Ser235/236}) ou IKK/NF- κ B (incluant I κ B α en abondance ou la phosphorylation de NF κ B^{Ser536}, IKK α / β ^{Ser177/181} et I κ B^{Ser32}) dans les muscles insulino-résistants. L'exercice n'empêche pas l'activation de l'une et l'autre de ces voies, comme le révèlent l'absence d'atténuation de la phosphorylation de protéines clés dans la voie IKK/NF- κ B (NF κ B^{Ser536}, IKK α / β ^{Ser177/181} et I κ B^{Ser32}), le maintien de l'abondance d'I κ B α ou la diminution de la phosphorylation des protéines clés dans la voie mTORC1 (mTOR^{Ser2448}, TSC2^{Ser939} et RPS6^{Ser235/236}). L'exercice induit plutôt une augmentation de la phosphorylation de deux protéines de la voie mTORC1 (PRAS40^{Thr246} et p70S6K^{Thr412}) dans les muscles stimulés par l'insuline, et ce, indépendamment du régime alimentaire. L'insulino-résistance induite par un bref HFD n'est pas attribuable à une plus grande activation des voies mTORC1 ou IKK/NF- κ B dans le muscle et l'amélioration de la sensibilité à l'insuline par l'exercice physique n'est pas due à la diminution de l'activation de ces voies dans le muscle. [Traduit par la Rédaction]

Keywords

physical activity; obesity; insulin resistance; Akt; inflammation

Introduction

One exercise session can induce a subsequent elevation in whole-body insulin sensitivity that is largely attributable to increased insulin-stimulated glucose uptake by skeletal muscle, and this health benefit can occur in both healthy and insulin-resistant individuals (Richter et al. 1982; Cartee and Holloszy 1990; Perseghin et al. 1996; Wojtaszewski et al. 2000; Arias et al. 2007; Cartee and Funai 2009; Funai et al. 2009; Pehmoller et al. 2012; Castorena et al. 2014). The mechanisms for improved insulin sensitivity after acute exercise have not been completely elucidated for healthy or insulin-resistant individuals.

A high-fat diet (HFD) for only 2–3 weeks can cause muscle insulin resistance in rodents (Turner et al. 2013; Castorena et al. 2014). Studying brief HFD offers an opportunity to identify the primary mechanisms for insulin resistance that precede obesity. We recently reported that brief HFD in rats caused insulin resistance for glucose uptake by isolated muscles, and in both low-fat diet (LFD) and HFD groups, acute exercise resulted in improved insulin-stimulated glucose uptake by isolated muscles (Castorena et al. 2014). The current study used the same diet and exercise protocols to provide further insights into the metabolic consequences of brief HFD and acute exercise.

Muscle lipid metabolites are potential triggers for insulin resistance, but few studies have assessed the effects of brief HFD. We measured muscle triacylglycerides to learn whether brief HFD was sufficient to increase muscle lipid accumulation. Because fatty acyl coenzyme A (CoA) species are proposed to induce insulin resistance (Hegarty et al. 2003), we also measured fatty acyl CoA species in muscle. We hypothesized that the HFD would increase fatty acyl CoA levels and exercise would attenuate this effect.

A lipid overload produced by high fat feeding may modify metabolism by altering various signaling pathways. The inhibitor of κ B kinase/nuclear factor κ B (IKK/NF- κ B) pathway (Shoelson et al. 2003) and the mammalian target of rapamycin complex 1 (mTORC1) pathway have been implicated in the development of insulin resistance (Um et al. 2006; Howell and Manning 2011). A novel aspect of the current study is that it assessed both of these pathways in muscle of rats after brief HFD compared with control rats maintained on a low-fat diet. Another novel aspect of the current study is that the influence of acute exercise on each pathway in muscle was evaluated in both normally insulin-sensitive and insulin-resistant rats. We hypothesized that (i) brief HFD would activate both pathways and (ii) exercise would attenuate these HFD effects.

Although insulin-stimulated Akt activity in muscle is not greater after acute exercise (Wojtaszewski et al. 2000; Fisher et al. 2002; Cartee and Funai 2009; Funai et al. 2009; Castorena et al. 2014), Akt's phosphorylation of its protein substrate, known as Akt substrate of 160 kDa (also called AS160 or TBC1D4), is elevated in muscle postexercise (Arias et al. 2007; Funai et al. 2009, 2010; Treebak et al. 2009; Pehmoller et al. 2012; Schweitzer et al. 2012; Castorena et al. 2014). Earlier research demonstrated that prior exercise did not alter the subsequent phosphorylation of 2 other Akt substrates (AS160's paralogue protein, known as TBC1D1, and glycogen synthase kinase 3, GSK3) in insulin-stimulated muscles (Wojtaszewski et al. 2000; Arias et al. 2007; Funai et al. 2009; Pehmoller et al. 2012). In this context, it is reasonable to ask whether AS160 is unique among Akt's many protein substrates or whether exercise also enhances the phosphorylation of other Akt substrates. Two Akt substrates were evaluated as part of the assessment of the mTORC1 pathway (proline-rich Akt substrate of 40 kDa, PRAS40, and tuberous sclerosis 2, TSC2). Accordingly, the current study provides novel insights into the effect of acute exercise on protein substrates of Akt.

In the current study, epitrochlearis muscles from rats (with or without acute swim exercise) were evaluated for several reasons. (i) The fiber type profile of the epitrochlearis is similar to the average fiber type profile of 76 different muscles or muscle parts of rats (Delp and Duan

1996; Allaf et al. 2002; Castorena et al. 2011). (ii) The epitrochlearis is a very thin muscle, making it well suited for ex vivo incubation (Wallberg-Henriksson 1987). (iii) The epitrochlearis is recruited by swim exercise, as evidenced by depletion of glycogen and activation of AMP-dependent protein kinase (Arias et al. 2007; Funai et al. 2009; Castorena et al. 2014). (iv) Because of its advantages, the rat epitrochlearis has been used to assess the effects of swim exercise on glucose uptake and insulin signaling in dozens of previous studies, so the results of additional studies using this muscle can be interpreted in the context of this extensive knowledge base. (v) Finally, the effect of swim exercise on insulin-stimulated glucose uptake by the rat epitrochlearis is reminiscent of results in rat hindlimb muscles after treadmill exercise (Richter et al. 1982) or in human muscle after stair climbing or knee extensor exercise (Perseghin et al. 1996; Wojtaszewski et al. 2000; Pehmoller et al. 2012).

Materials and methods

Materials

Materials for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting were from Bio-Rad (Hercules, Calif., USA). The following kits and reagents were from Millipore (Billerica, Mass., USA): MILLIPLEX MAP Cell Signaling Buffer and Detection Kit (No. 48-602MAG), MILLIPLEX MAP Akt/mTOR Phosphoprotein panel (No. 48-611MAG, which included phosphorylated mammalian target of rapamycin, p-mTOR^{Ser2448}; phosphorylated ribosomal protein S6, pRPS6^{Ser235/236}; phosphorylated p70 ribosomal protein S6 kinase, p-p70S6K^{Thr412}; phosphorylated glycogen synthase kinase 3 alpha and beta, pGSK3 α ^{Ser21} and pGSK3 β ^{Ser9}; phosphorylated phosphatase and tensin homologue deleted on chromosome 10, pPTEN^{Ser380}; and phosphorylated tuberous sclerosis 2, pTSC2^{Ser939}), MILLIPLEX MAP NF- κ B panel (No. 48-630MAG, which included phosphorylated nuclear factor κ B, pNF- κ B^{Ser536}; phosphorylated inhibitor of κ B kinase α / β , pIKK α / β ^{Ser177/181}; phosphorylated inhibitor of κ B, pI κ B^{Ser32}; phosphorylated Fas-associated death domain, pFADD^{Ser194}; c-Myc; and tumor necrosis factor receptor 1, TNFR1), MILLIPLEX MAP Total I κ B α MAPmate (No. 46-644), and Luminata Forte Western HRP substrate (No. WBLUR0500). Anti-pPRAS40^{Thr246} (No. 2997) was from Cell Signaling Technology (Boston, Mass., USA). The bicinchoninic acid (BCA) protein assay (No. 23231) was purchased from Thermo Fisher Scientific (Pittsburg, Penn., USA). [³H]2-Deoxy-D-glucose (2-DG) and [¹⁴C]mannitol were from PerkinElmer (Waltham, Mass., USA). The insulin ELISA kit (80-INSRTU-E01) was from ALPCO Diagnostics (Salem, N.H., USA). An Accu-Chek glucose meter was purchased from Roche (Indianapolis, Ind., USA).

Animal treatment

Animal care methods were approved by the University Committee on Use and Care of Animals at the University of Michigan. Male Wistar rats (initial body weight of approximately 200 to 250 g; Harlan Laboratories, Indianapolis, Ind., USA) were fed either a typical rodent chow (low-fat diet, LFD: 14% kcal fat, 58% kcal carbohydrate, 28% kcal protein; LabDiet No. 5001, PMI Nutrition International, Brentwood, Mo., USA) or a high-fat diet (HFD: 60% kcal fat, 20% kcal carbohydrate, 20% kcal protein; D12492, Research

Diets, New Brunswick, N.J., USA) and water ad libitum for 2 weeks. The animals were fasted at ~1900 on the night before exercise was performed. Beginning at ~0700, exercised rats swam in a barrel filled with water (35 °C, ~45 cm depth, 6 rats per barrel, 3 rats from each diet) for 4 × 30 min with 5 min rest between bouts (Castorena et al. 2014). After exercise, the rats were dried and provided water ad libitum. Blood was collected from exercised animals at 3 h postexercise (3hPEX) concomitantly with blood collection from time-matched sedentary controls to measure plasma glucose and insulin. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as follows: plasma glucose (mg/dL) × plasma insulin (μU/mL)/405. Rats were then anesthetized (intraperitoneal sodium pentobarbital, 50 mg/kg), and epitrochlearis muscles were isolated from the anesthetized rats.

Muscle incubations

Paired epitrochlearis muscles were incubated in vials with continuous gassing (95% O₂/5% CO₂) and shaking in a heated (35 °C) water bath to determine insulin-independent (no insulin) and insulin-dependent (plus insulin) glucose uptake. The muscles underwent a 2-step incubation. In the first step, each muscle was placed for 30 min in a vial containing 2 mL of Krebs–Henseleit buffer with 0.1% bovine serum albumin (KHB+BSA), 2 mmol/L sodium pyruvate, 6 mmol/L mannitol, and no insulin or 100 μU/mL insulin. In the second step, each muscle was placed for 20 min in a vial containing 2 mL of KHB+BSA, the same insulin concentration as in the previous step, 1 mmol/L [³H]2-DG (2.25 mCi/mmol), and 9 mmol/L [¹⁴C]mannitol (0.022 mCi/mmol). Subsequently, the muscles were rapidly blotted and then freeze-clamped and stored at –80 °C.

Homogenization of muscles used for glucose uptake and protein analyses

Frozen muscles that had been used for 2-DG uptake were weighed and then homogenized in ice-cold lysis buffer (20 mmol/L Tris-HCl, 150 mmol/L NaCl, 1% Triton X-100, 1 mmol/L Na₃VO₄, 1 mmol/L EDTA, 1 mmol/L EGTA, 2.5 mmol/L sodium pyrophosphate, 1 mmol/L β-glycerophosphate, 1 μg/mL leupeptin, and 1 mmol/L phenylmethylsulfonyl fluoride; 1 mL/muscle) using a TissueLyser II homogenizer (Qiagen Inc., Valencia, Calif., USA). Homogenates were rotated for 1 h at 4 °C and then centrifuged at 10 000g for 10 min (4 °C). The supernatant was collected for measurement of total protein concentration by BCA assay using the manufacturer's protocol (Pierce Biotechnology, No. 23227). The remaining supernatant was stored (–80 °C) until subsequent analysis.

Glucose uptake

Aliquots of supernatants were added to vials containing scintillation cocktail, ³H and ¹⁴C disintegrations per minute were measured by a scintillation counter, and 2-DG uptake was calculated as previously described (Cartee and Bohn 1995).

Immunoblotting

Equal protein concentrations for muscle samples, as determined using the BCA assay, were boiled (5–7 min) in SDS loading buffer, separated using SDS-PAGE, and transferred to nitrocellulose membranes. The membranes were incubated with the appropriate primary and

secondary antibodies. Enhanced chemiluminescence of protein bands was quantified by densitometry (Alpha Innotech, San Leandro, Calif., USA) (Castorena et al. 2011). Individual values were normalized to the mean value of all samples on the membrane.

Multiplex analysis

Multiplex assays (Luminex L200 instrument and xPONENT software, version 3.1; Luminex, Austin, Tex., USA) for mTOR^{Ser2448}, RPS6^{Ser235/236}, p70S6K^{Thr412}, GSK3 α ^{Ser21}, GSK3 β ^{Ser9}, PTEN^{Ser380}, and TSC2^{Ser939} (Sharma et al. 2012) were performed on muscle lysates containing equal amounts of total protein. In a second Luminex assay, muscle homogenates were used to measure pNF- κ B^{Ser536}, pIKK α / β ^{Ser177/181}, pI κ B^{Ser32}, c-Myc, pFADD^{Ser194}, and TNFR1. A singleplex assay was performed for I κ B α .

Muscle lipid analysis

Three hours following the exercise bouts, epitrochlearis muscles were isolated from anesthetized rats, immediately freeze-clamped, and stored (-80°C) for lipid analysis. The University of Michigan Molecular Phenotyping Core performed lipid extraction and analysis. Briefly, muscles were powdered under liquid nitrogen and homogenized in ice-cold 1 mol/L NaCl prior to lipid extraction (Morrison and Smith 1964; Kasumov et al. 2010). Triacylglycerides (TAG) were isolated via thin-layer chromatography (TLC) using hexane–diethyl ether–acetic acid (70:30:1.5, v/v) as the developing solvent. The TLC powders of the TAG bands corresponding to the retention time of authentic standard (triolein) were extracted with ether and the lipids were subjected to transesterification with boron trifluoride–methanol. The methyl esters of TAG were then analyzed on a gas chromatography column with a flame ionization detector (Morrison and Smith 1964). The fatty acid components were identified by comparing their retention times with those of standard methyl esters run with each sample. The components were quantified with respect to heptadecanoyl (C_{17}) methyl ester, which was added in a known amount to each sample as an internal standard. Acetyl CoA was extracted and analyzed by high-performance liquid chromatography and tandem mass spectrometry and then quantified by electrospray ionization–multiple reaction monitoring–mass spectrometry (Kasumov et al. 2010).

Statistics

Two-way ANOVA was used to compare means of more than two groups (SigmaPlot, version 11.0; Jandel Scientific, San Rafael, Calif., USA). Tukey post hoc analysis was used to identify the source of significant variance (SigmaPlot). Data are expressed as means $\pm$ SE. A P value <0.05 was considered statistically significant.

Results

Plasma glucose, plasma insulin, and HOMA-IR

For plasma glucose (Fig. 1A), there was a significant main effect of exercise (sedentary $>$ 3hPEX; $P < 0.01$) and a significant interaction between diet and exercise ($P < 0.05$). Post hoc analysis revealed that plasma glucose was significantly greater ($P < 0.05$) for sedentary HFD rats than for sedentary LFD rats, but the diet-related difference in glucose was eliminated at 3hPEX, with plasma glucose values for both 3hPEX LFD and 3hPEX HFD

rats being significantly lower ($P < 0.05$) than those for diet-matched sedentary controls. For plasma insulin (Fig. 1B), there were significant main effects of both diet (HFD > LFD; $P < 0.01$) and exercise (sedentary > 3hPEX; $P < 0.01$). Post hoc analysis indicated that within the sedentary groups, HFD rats had significantly greater ($P < 0.05$) plasma insulin than LFD rats; in contrast, within the 3hPEX groups, plasma insulin was not different between diet groups. Plasma insulin was significantly reduced ($P < 0.05$) in 3hPEX HFD rats compared with sedentary HFD rats. For HOMA-IR (Fig. 1C), there were significant main effects of diet (HFD > LFD; $P < 0.01$) and exercise (sedentary > 3hPEX; $P < 0.01$). In the sedentary groups, HFD rats had significantly greater ($P < 0.05$) HOMA-IR than LFD rats, but in the 3hPEX cohorts, HOMA-IR did not differ between diet groups. For both LFD and HFD, HOMA-IR was significantly reduced ($P < 0.05$) for the 3hPEX group compared with the sedentary group.

Glucose uptake

The muscle glucose uptake values were reported in an earlier publication (Castorena et al. 2014). For muscles incubated without insulin, there was a significant main effect of exercise (3hPEX > sedentary; $P < 0.05$) on 2-DG uptake. Post hoc analysis revealed that 2-DG uptake was higher in muscle of 3hPEX LFD rats than in muscle of sedentary LFD rats. There were significant ($P < 0.01$) main effects of diet (LFD > HFD) and exercise (3hPEX > sedentary) for 2-DG uptake with insulin. Post hoc analysis indicated that 2-DG uptake with insulin was higher for 3hPEX LFD rats than for all the other groups ($P < 0.05$). Post hoc analysis also revealed greater 2-DG uptake with insulin ($P < 0.05$) in the 3hPEX HFD group compared with the sedentary HFD group.

Protein analysis

For p-p70S6K^{Thr412} (Fig. 2A) in muscles incubated with insulin, there was a significant main effect of exercise (3hPEX > sedentary; $P < 0.05$). For pPTEN^{Ser380} (Fig. 2B) in muscles incubated either with or without insulin, there was a significant main effect of diet (HFD > LFD; $P < 0.01$), and post hoc analysis revealed that within both the sedentary and 3hPEX groups, the HFD values exceeded the LFD values ($P < 0.05$). Regardless of insulin concentration, neither diet nor exercise significantly altered p-mTOR^{Ser2448}, pTSC2^{Ser939}, pRPS6^{Ser235/236} (Figs. 2C–2E), pGSK3 α ^{Ser21}, or pGSK3 β ^{Ser9} (not shown). For pPRAS40^{Thr246} (Fig. 3) in muscles incubated with insulin, there was a significant main effect of exercise (3hPEX > sedentary; $P < 0.01$), and post hoc analysis revealed that 3hPEX LFD values exceeded sedentary LFD values ($P < 0.05$).

For TNFR1 (Fig. 4A) in muscles incubated with insulin and c-Myc (not shown) in muscles incubated either with or without insulin, there were small but significant main effects of exercise (sedentary > 3hPEX; $P < 0.05$). For pIKK α /p β ^{Ser177/181} (Fig. 4B) in muscles incubated without insulin, there was a significant main effect of diet (LFD > HFD; $P < 0.01$), and post hoc analysis indicated that within the 3hPEX groups, LFD values exceeded HFD values; in insulin-treated muscles, there was a significant ($P < 0.05$) diet $\times$ exercise interaction. For pI κ B^{Ser32} (Fig. 4C) in muscles incubated with insulin, there was a significant main effect of diet (LFD > HFD, $P < 0.05$), and post hoc analysis indicated that within the 3hPEX groups, the LFD values exceeded the HFD values. For pNF- κ B^{Ser536} (Fig.

4D), I κ B α (Fig. 4E), and pFADD^{Ser194} (not shown), there were no significant effects of either diet or exercise, regardless of insulin concentration.

Muscle lipids

There were significant main effects of diet (HFD > LFD; $P < 0.05$) for muscle TAG containing 18:0, 18:1 (n-9), and 20:2 (Table 1), but post hoc analysis did not reveal any further differences. For TAG containing 20:1, there was a significant main effect of exercise (sedentary > 3hPEX; $P < 0.01$), and post hoc analysis indicated that sedentary HFD values exceeded 3hPEX LFD values ($P < 0.05$). There was a significant main effect of exercise (sedentary > 3hPEX; $P < 0.05$) on 16:0 CoA in muscle (Fig. 5), but post hoc analysis did not reveal any further differences.

Discussion

The brief HFD produced whole-body insulin resistance, as reflected by higher plasma insulin, plasma glucose, and HOMA-IR values for HFD rats compared with LFD rats, and exercise improved whole-body insulin sensitivity in both diet groups. We previously reported that brief HFD reduced insulin-stimulated glucose uptake in isolated epitrochlearis muscles, and exercise led to greater insulin-stimulated glucose uptake in muscles from both diet groups (Castorena et al. 2014). In the current study, exercise eliminated the HFD-related effects on insulin, glucose, and HOMA-IR values, but we previously found that exercise did not eliminate the HFD-related effect on insulin-stimulated glucose uptake by muscle (Castorena et al. 2014). Although exercise elevated insulin-stimulated glucose uptake by isolated muscles of the HFD group, the values for the 3hPEX HFD group were similar to those for the sedentary LFD group and were still significantly lower than those for the 3hPEX LFD group (Castorena et al. 2014). One possible explanation for the difference between the muscle and whole-body results is that fasting insulin, glucose, and HOMA-IR values are greatly influenced by hepatic glucose production and not only by insulin-stimulated glucose uptake of muscle.

As expected, the HFD protocol resulted in greater muscle levels of several species of TAG containing 18:0, 18:1 (n-9), and 20:2. The only significant effect of exercise on TAG composition was a reduction in TAG with 20:1, which is a less abundant fatty acid associated with TAG that was not elevated by HFD. It is widely believed that increased muscle TAG levels do not directly cause insulin resistance; however, the accumulation of other lipids, including ceramides and/or diacylglycerides, is often linked to the development of insulin resistance. However, we recently reported that neither 2 weeks of HFD nor acute exercise significantly altered muscle levels of ceramide or diacylglyceride species in the epitrochlearis of rats (Castorena et al. 2014). In the current study, 16:0 CoA values were reduced after exercise compared with the sedentary condition, and it is possible that this reduction has a role in the previously reported exercise-induced increase in insulin-stimulated glucose uptake (Castorena et al. 2014). However, 16:0 CoA levels did not differ between the two diet groups at 3hPEX, so this lipid species does not appear to account for the greater insulin-stimulated glucose uptake previously observed in the 3hPEX LFD group compared with the 3hPEX HFD group (Castorena et al. 2014).

Insulin, IGF-1, energy status, and amino acids are among the various inputs that contribute to the complex regulation of the mTORC1 pathway. The regulation and function of this pathway have been recently reviewed in great detail (Howell and Manning 2011; Laplante and Sabatini 2012; Huang and Fingar 2014). Briefly, insulin causes activation of phosphatidylinositol-3-kinase (PI3K), and PI3K activation leads to Akt activation. Akt, in turn, phosphorylates TSC2, thereby eliminating TSC2's inactivation of Rheb, and the release of Rheb inhibition leads to stimulation of mTORC1. The multiprotein complex mTORC1 includes the mTOR catalytic subunit, PRAS40, raptor, and at least 4 other proteins. Phosphorylation of PRAS40 by Akt inhibits binding of PRAS40 to mTORC1, and the release of PRAS40 appears to favor mTORC1 activation. Increased mTORC1 activity ultimately results in the phosphorylation of multiple protein substrates, including p70S6K; when activated, p70S6K phosphorylates its substrate RPS6. Under some conditions, p70S6K can also phosphorylate multiple serine residues of insulin receptor substrate-1 (IRS-1) as well as the serine phosphorylate rictor (a component of the mTORC2 complex that activates Akt). The link between these outcomes and insulin resistance has been recently reviewed (Rivas et al. 2009a; Copps and White 2012).

There were no significant effects of HFD on any of the proteins that would be expected to favor greater mTORC1 activity. HFD resulted in a small but significant increase in pPTEN^{Ser380}. This post-translational modification can reduce the lipid phosphatase activity of PTEN, which dephosphorylates phosphatidylinositol 3,4,5-trisphosphate (Georgescu 2010). In this context, the small HFD-related increase in pPTEN^{Ser380} could potentially favor greater PI3K-related signaling rather than causing insulin resistance. However, we previously reported that the same HFD protocol did not result in altered IRS-1–PI3K activity in muscle (Castorena et al. 2014). Some studies have shown that long-term HFD (8 to 16 weeks) leads to increased phosphorylation of mTOR, p70S6K, and/or RPS6 in muscle of rats or mice (Le Bacquer et al. 2007; Rivas et al. 2009b), whereas another study showed that long-term HFD (24 weeks) did not alter phosphorylation of mTOR in muscles of rats (Li et al. 2013). Neither HFD nor exercise altered phosphorylation of GSK3α/β in the current study, but an earlier study reported that long-term HFD (15 weeks) led to reduced GSK3α/β phosphorylation in rat muscle (Henriksen et al. 2008). Although long-term HFD can influence mTORC1 and GSK3α/β activation in rodent muscle, the HFD-induced insulin resistance observed in the current study did not appear to be attributable to enhanced mTORC1 activation or attenuated GSK3α/β phosphorylation.

There were no significant effects of exercise on p-mTOR^{Ser2448}, pTSC2^{Ser939}, or pRPS6^{Ser235/236}. However, exercise induced greater pPRAS40^{Thr246} and p-p70S6K^{Thr412} in insulin-stimulated muscles from both diet groups. It was previously reported that phosphorylation of mTOR, p70S6K, and/or RPS6 in rat muscle was increased immediately after acute treadmill exercise (1 to 2 h duration) (Edgett et al. 2013; Ogasawara et al. 2014) and that these increases were no longer evident at 1 or 3 h postexercise. In contrast, another study of acute treadmill exercise (1 h duration) did not detect altered phosphorylation of mTOR or p70S6K in rat muscle either immediately or 2 h postexercise (de Souza et al. 2013). In addition, an earlier study of acute swim exercise (3 h duration) did not find altered phosphorylation of mTOR, p70S6K, or RPS6 in rat muscle assessed immediately, 0.5 h, or 1.5 h postexercise (Morrison et al. 2008). Consistent with the current study, none of the

previous studies provided evidence of attenuated mTORC1 signaling at any time after exercise. The results of the current study suggest that improvements in insulin sensitivity after acute exercise, regardless of diet, were unlikely to be caused by reduced activation of the mTORC1 pathway in muscle. A limitation of the current study is that mTORC1 activation was studied only at 3–4 h postexercise, but it is notable that none of the earlier studies found reduced mTORC1 activity at earlier time points after acute exercise. However, it should also be noted that none of the earlier studies that evaluated times prior to 3–4 h postexercise included insulin-resistant, HFD-fed rats.

The results for pPRAS40^{Thr246} demonstrate that AS160 is not the only Akt substrate with enhanced phosphorylation in insulin-stimulated muscles several hours postexercise. Furthermore, the effect of prior exercise on subsequent phosphorylation is found not only for proteins that are substrates of Akt. PDK1, rather than Akt, has been identified as a kinase that phosphorylates p70S6K at Thr412 (Balendran et al. 1999; Frodin et al. 2002), and there is also a significant exercise-related increase in p-p70S6K^{Thr412} in insulin-stimulated muscles. It is unclear whether there is any overlap in the mechanisms that account for greater phosphorylation of PRAS40, p70S6K, and AS160 several hours postexercise. It is also uncertain whether there are functional consequences of the exercise-induced increases in pPRAS40^{Thr246} and/or p-p70S6K^{Thr412}. Phosphorylation of p70S6K at Thr412 is reportedly important for the enzymatic activity of p70S6K (Alessi et al. 1998; Weng et al. 1998; Beigh et al. 2012), and p70S6K is a positive regulator of protein synthesis. PRAS40 binds to raptor, which is part of mTORC1, and Akt-dependent phosphorylation of PRAS40 at Thr246 allows greater phosphorylation of PRAS40 at Ser183 by mTOR, which in turn releases PRAS40 from binding to raptor and eliminates PRAS40's inhibition of mTORC1 activity (Wang et al. 2012). There are differing results regarding the possible influence of PRAS40 on insulin signaling and insulin-stimulated glucose uptake. In cultured myocytes, PRAS40 knockdown was accompanied by reduced insulin-stimulated Akt activation and glucose uptake (Wiza et al. 2013), and overexpression of PRAS40 increased insulin-stimulated Akt phosphorylation and glucose uptake in cultured human skeletal muscle cells (Wiza et al. 2014). However, others reported that neither increasing nor decreasing PRAS40 expression resulted in altered Akt activation in HEK293 cells (Fonseca et al. 2007).

The potential role of the IKK/NF- κ B pathway as a mediator of insulin resistance and metabolic dysregulation has been recently reviewed in detail (Glass and Olefsky 2012; Tornatore et al. 2012). TNF α and other proinflammatory cytokines can engage their respective receptors (e.g., TNFR1 for TNF α), leading to the phosphorylation and activation of IKK (Hayden and Ghosh 2012). A wide variety of signals, including lipids and reactive oxygen species, can also induce the activation of IKK. In the inactive state, NF- κ B is bound to I κ B, and this protein complex is mainly sequestered in the cytosol. Activated IKK can phosphorylate I κ B, which results in I κ B's ubiquitination and subsequent degradation. Dissociation from I κ B allows free NF- κ B in the cytosol to undergo ubiquitination and phosphorylation, followed by translocation to the nucleus. In the nucleus, NF- κ B binds to specific promoters and induces the expression of target genes with diverse effects on signaling and metabolism. In addition, activated IKK can phosphorylate IRS-1 at multiple serine residues, which interferes with IRS-1's role in the insulin signaling pathway (Osborn and Olefsky 2012).

The results for the IKK/NF- κ B pathway in the current study suggest that neither the HFD-related insulin resistance nor the exercise-induced increase in insulin sensitivity were secondary to changes in this pathway. There were no significant main effects of either diet or acute exercise on muscle levels of pNF- κ B^{Ser536} or I κ B α . In muscles incubated without insulin, there was a small but significant effect of diet on pIKK α / β ^{Ser177/181}, with the HFD animals having lower values than the LFD animals (suggesting reduced activation in the HFD group). Similarly, in muscles incubated with insulin, there was a small but significant reduction in pI κ B^{Ser32} for HFD versus LFD rats.

Several earlier studies assessed the effects of long-term HFD and/or exercise on the IKK/NF- κ B pathway in rat muscle. Ropelle et al. (2006) found that HFD for 3 months resulted in lower muscle levels of I κ B α in rats, indicative of activation of the IKK/NF- κ B pathway. They also reported that a prolonged (6 h duration) swim exercise session by HFD-fed rats led to an increase in muscle I κ B α levels at 16 h postexercise. Pauli et al. (2010) reported that muscle I κ B α levels were reduced in 27-month-old rats, and this age-related decline was reversed 16 h after a single session of swim exercise (3 h duration). In contrast to these results, other studies have demonstrated that the IKK/NF- κ B pathway can be activated in rat muscles during and shortly after acute exercise. Immediately after 1 h of treadmill exercise by rats that were consuming a LFD, muscle phosphorylation of I κ B α was increased and I κ B α abundance was reduced, and these exercise-induced changes were reversed by 4 h postexercise, with no exercise effect evident at 24 or 48 h postexercise (Ji et al. 2004). Muscle pIKK was increased immediately postexercise, but the time course for reversal of this effect after exercise was not reported (Ji et al. 2004). Ho et al. (2005) found that treadmill running for 5, 15, 30, or 60 min did not alter muscle I κ B α or I κ B β levels determined immediately, 1 h, or 3 h postexercise. Muscle pIKK α / β was significantly increased only immediately after 15 min of exercise, with no effect at 1 or 3 h postexercise. The lack of exercise-related changes at 3 to 4 h postexercise in these earlier studies of rats is similar to observations for the 3hPEX groups in the current study.

The current study focused on a single time point (~3 to 4 h after exercise) that was selected because insulin-stimulated glucose uptake has been repeatedly shown to be elevated in the muscle of rats at this time (Wallberg-Henriksson et al. 1988; Cartee et al. 1989; Cartee and Holloszy 1990; Fisher et al. 2002; Arias et al. 2007; Funai et al. 2009, 2010; Castorena et al. 2014). The specific diet and exercise protocols and the time of muscle sampling relative to completion of exercise may account for the differences in results among various studies, and it remains possible that some of the results would have been different at another time point after exercise or if different dietary or exercise protocols had been used. Another limitation of the current study is that there was no analysis of the subcellular localization of proteins or lipids.

In conclusion, brief HFD that resulted in accumulation of muscle TAG species and produced whole-body and muscle insulin resistance did not appear to activate either the IKK/NF- κ B pathway or the mTORC1 pathway in muscle. A novel aspect of this study is the characterization of the effects of brief HFD on the IKK/NF- κ B and mTORC1 pathways in muscle. Another novel aspect of the study is the demonstration that the acute exercise-induced enhancement of insulin sensitivity in rats (LFD or brief HFD) was not attributable

to attenuation of either pathway in muscle. The current study is also the first to reveal greater site-specific phosphorylation of PRAS40 and p70S6K in insulin-stimulated muscles after acute exercise. Future research is needed to determine whether a common mechanism is responsible for the effect of prior exercise on the phosphorylation of these proteins, as well as AS160 (Castorena et al. 2014), and to assess the functional consequences of these exercise-induced outcomes.

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Fig. 1.

Plasma was obtained from low-fat diet (LFD) and high-fat diet (HFD) rats at 3 h postexercise (3hPEX) and time-matched sedentary controls. (A) Plasma glucose. (B) Plasma insulin. (C) HOMA-IR. Data were analyzed by 2-way ANOVA. Tukey post hoc analysis was performed to identify the source of significant variance. *, $P < 0.05$, LFD versus HFD, within sedentary. †, $P < 0.05$, for 3hPEX versus sedentary within each diet group. Values are means $\pm$ SE; $n = 36$ per group. D $\times$ E, diet $\times$ exercise.

Fig. 2.

Paired epitrochlearis muscles from low-fat diet (LFD) and high-fat diet (HFD) rats at 3 h postexercise (3hPEX) and time-matched sedentary controls following incubations with and without insulin. Phosphorylation of (A) p70 ribosomal protein S6 kinase at Thr412 (p-p70S6K^{Thr412}), (B) phosphatase and tensin homologue deleted on chromosome 10 at Ser380 (pPTEN^{Ser380}), (C) mammalian target of rapamycin at Ser2448 (p-mTOR^{Ser2448}), (D) tuberous sclerosis 2 at Ser939 (pTSC2^{Ser939}), and (E) ribosomal protein S6 at Ser235/236 (pRPS6^{Ser235/236}). Because these proteins were measured by multiplex analysis, there are no representative blots to display. Data were analyzed by 2-way ANOVA within each insulin level. *, $P < 0.05$ for LFD versus HFD within the same activity and insulin group. Values are means $\pm$ SE for sedentary LFD ($n = 19$), sedentary HFD ($n = 20$), 3hPEX LFD ($n = 19$), and 3hPEX HFD ($n = 18$). D $\times$ E, diet $\times$ exercise.

Fig. 3.

Paired epitrochlearis muscles from low-fat diet (LFD) and high-fat diet (HFD) rats at 3 h postexercise (3hPEX) and time-matched sedentary controls following incubations with and without insulin. Immunoblot shows phosphorylation of proline-rich Akt substrate of 40 kDa at Thr246 (pPRAS40^{Thr246}). Data were analyzed by 2-way ANOVA within each insulin level, and Tukey post hoc analysis was performed to identify the source of significant variance. *, $P < 0.05$ for 3hPEX LFD versus LFD SED with insulin. Values are means $\pm$ SE; $n = 8$ per group. D $\times$ E, diet $\times$ exercise; SED, sedentary.

Fig. 4.

Paired epitrochlearis muscles from low-fat diet (LFD) and high-fat diet (HFD) rats at 3 h postexercise (3hPEX) and time-matched sedentary controls following incubations with and without insulin. (A) Tumor necrosis factor receptor one (TNFR1). (B) Inhibitor of κ B kinase alpha/beta phosphorylated at Ser177/181 (pIKK α / β ^{Ser177/181}) measured in paired epitrochlearis muscles 3hPEX. (C) Inhibitor of κ B phosphorylated at Ser32 (pI κ B^{Ser32}) measured in paired epitrochlearis muscles 3hPEX. (D) Nuclear factor κ B phosphorylated at Ser536 (pNF- κ B^{Ser536}). (E) Inhibitor of κ B alpha (I κ B α). Because these proteins were measured by multiplex analysis, there are no representative blots to display. Data were analyzed by 2-way ANOVA within each insulin level. Tukey post hoc analysis was performed to identify the source of significant variance. *, $P < 0.05$, LFD greater than HFD within the same insulin and exercise group. Values are means $\pm$ SE; $n = 8$ per group. D $\times$ E, diet $\times$ exercise.

Fig. 5.

Epitrochlearis acetyl CoA and acyl CoA species (ng/mg) for low-fat diet (LFD) and high-fat diet (HFD) rats at 3 h postexercise (3hPEX) and time-matched sedentary controls. Data were analyzed by 2-way ANOVA. Tukey post hoc analysis was performed to identify the source of significant variance. Values are means $\pm$ SE for acetyl CoA (LFD sedentary (SED), $n = 8$; HFD SED, $n = 6$; LFD 3hPEX, $n = 6$; HFD 3hPEX, $n = 7$), 16:0 CoA (LFD SED, $n = 8$; HFD SED, $n = 7$; LFD 3hPEX, $n = 7$; HFD 3hPEX, $n = 7$), and 18:1 CoA (LFD SED, $n = 8$; HFD SED, $n = 7$; LFD 3hPEX, $n = 9$; HFD 3hPEX, $n = 8$). D $\times$ E, diet $\times$ exercise.

Table 1

Fatty acid content of epitrochlearis triacylglycerides (TAG; nmol/mg wet muscle mass) for low-fat diet (LFD) and high-fat diet (HFD) rats at 3 h postexercise (3hPEX) and time-matched sedentary (SED) controls.

	14:0	14:1	16:0	16:1	18:0 [*]	18:1 (n-7)	18:1 (n-9) [*]	18:2	18:3 (n-3)	18:3 (n-6)	20:0	20:1 [*]	20:2 [†]	20:3	20:4	22:0	22:1
LFD SED	70.0±19.3	8.3±2.1	1849.0±242.9	163.6±28.8	757.8±65.7	251.3±20.0	2718.9±344.4	2103.6±274.5	66.1±14.6	4.3±0.9	16.2±1.3	43.5±4.6	38.2±4.5	11.4±1.4	61.9±6.7	6.0±0.4	1.9±0.4
HFD SED	107.7±22.1	5.8±1.8	2663.4±372.8	208.7±36.4	1152.0±134.3	269.9±24.7	4670.6±618.6	2849.4±376.4	95.3±13.9	5.3±0.9	17.5±1.1	50.9±7.9 [‡]	61.3±7.5	11.4±1.3	56.4±6.2	8.1±2.6	1.6±0.5
LFD 3hPEX	73.5±25.5	9.6±1.0	1983.1±518.7	157.6±40.7	814.1±187.1	230.4±37.1	3068.7±861.6	2280.8±634.7	72.1±19.0	3.7±1.1	18.0±3.3	21.6±5.5	37.6±9.2	10.5±2.2	48.9±6.7	12.2±5.6	3.0±1.0
HFD 3hPEX	108.9±31.3	8.0±1.3	2788.8±656.2	205.0±52.3	1126.3±223.5	239.9±33.9	5057.1±1174.1	3045.4±684.9	96.2±21.7	4.5±1.4	17.3±2.4	35.9±9.0	62.6±13.2	10.7±2.1	51.2±9.8	5.6±0.4	3.2±0.4

Note: Values are means ± SE; *n* = 5–9 per group. Data were analyzed by 2-way ANOVA. Tukey post hoc analysis was performed to identify the source of significant variance.

^{*} HFD SED, *P* < 0.05 for main effect of exercise.

[†] *P* < 0.05 for main effect of diet.

[‡] *P* < 0.05, HFD SED greater than LFD 3hPEX.