Reduced malonyl-CoA content in recovery from exercise correlates with improved insulin-stimulated glucose uptake in human skeletal muscle

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Froesig C, Roepstorff C, Brandt N, Maarbjerg SJ, Birk JB, Wojtaszewski JF, Richter EA, Kiens B. Reduced malonyl-CoA content in recovery from exercise correlates with improved insulin-stimulated glucose uptake in human skeletal muscle. Am J Physiol Endocrinol Metab 296: E787–E795, 2009. First published February 3, 2009; doi:10.1152/ajpendo.90556.2008.—This study evaluated whether improved insulin-stimulated glucose uptake in recovery from acute exercise coincides with reduced malonyl-CoA (MCoA) content in human muscle. Furthermore, we investigated whether a high-fat diet [65 energy-% (Fat)] would alter the content of MCoA and insulin action compared with a high-carbohydrate diet [65 energy-% (CHO)]. After 4 days of isocaloric diet on two occasions (Fat/CHO), 12 male subjects performed 1 h of one-legged knee extensor exercise (~80% peak workload). Four hours after exercise, insulin-stimulated glucose uptake was determined in both legs during a euglycemic-hyperinsulinemic clamp. Muscle biopsies were obtained in both legs before and after the clamp. Four hours after exercise, insulin-stimulated glucose uptake was improved (~70%, P < 0.001) independent of diet composition and despite normal insulin-stimulated regulation of insulin receptor substrate-1-associated phosphatidylinositol 3-kinase, Akt, GSK-3, and glycogen synthase. Interestingly, exercise resulted in a sustained reduction (~20%, P < 0.05) in MCoA content 4 h after exercise that correlated (r = 0.65, P < 0.001) with improved insulin-stimulated glucose uptake. Four days of Fat diet resulted in an increased content of intramyocellular triacylglycerol (P < 0.01) but did not influence muscle MCoA content or whole muscle insulin-stimulated glucose uptake. However, at the muscular level proximal insulin signaling and insulin-stimulated glucose uptake appeared to be compromised, although to a minor extent, by the Fat diet. Collectively, this study indicates that reduced muscle MCoA content in recovery from exercise may be part of the adaptive response leading to improved insulin action on glucose uptake after exercise in human muscle.

AMP-activated protein kinase; acetyl coenzyme A carboxylase; intramuscular triacylglycerol; Akt; diet

MALONYL COENZYME A (MCoA) is an allosteric inhibitor of carnitine palmitoyltransferase (CPT) I, the rate-limiting enzyme controlling transfer of long-chain fatty acyl-CoAs (FACoAs) into the mitochondria for oxidation (28, 29, 40). Accordingly, MCoA is considered an inhibitor of mitochondrial fat oxidation in vivo. On the basis of rapid changes in concentration of MCoA in response to altered fuel supply or energy expenditure in skeletal muscle, MCoA has been referred to as an important fuel sensing and signaling molecule (40). Thus in both rodent and human muscle insulin stimulation leads to MCoA accumulation, likely as a result of an increased cytosolic concentration of citrate, allosterically activating acetyl-CoA carboxylase-β (ACC-β), the enzyme responsible for MCoA synthesis from acetyl coenzyme A (5, 43, 45). This subsequently decreases lipid oxidation. Conversely, in response to exercise (50) or electrically induced contractions (15, 49) in rodents, the concentration of MCoA is reduced within minutes by a mechanism involving both inhibition of ACC-β and activation of malonyl-CoA decarboxylase (MCD). These cellular adaptations to exercise are believed to stimulate lipid oxidation and to result at least in part from activation of the AMP-activated protein kinase (AMPK) during contractions (42, 49, 51).

In contrast to these observations in rodents, the role of MCoA in acute metabolic regulation during exercise in human muscle is less clear (13, 38, 49).

Apart from regulation of substrate oxidation, an association between MCoA concentration and insulin action on glucose uptake has been proposed. On theoretical grounds, it has been argued that sustained or chronically increased concentrations of MCoA may lead to accumulation of diacylglycerol (DAG) or ceramides when FACoA oxidation is reduced (13, 39, 47). This results in activation of novel protein kinase Cs (nPKCs) capable of serine phosphorylation of proximal insulin signaling components, thereby reducing proximal insulin signaling capacity (44). Support for this theory is the finding of increased levels of MCoA and DAG concomitant with altered nPKC regulation in muscle in a range of insulin-resistant rodent models, including fadfa and Goto-Kakizaki (GK) rats, and in rats in response to fat feeding or denervation as reviewed by Saha and Ruderman (41).

In contrast to these more chronic adaptations, little is known about the effect of acute alterations in MCoA content on insulin action in muscle. In cultured C2C12 myotubes, polyamine-mediated delivery of MCoA or exposure to dichloroacetate (reported to increase MCoA levels) resulted in prevention of insulin-stimulated glucose uptake, while three unrelated inhibitors of ACC-β acutely increased insulin-stimulated glucose uptake (32). Interestingly, none of these interventions resulted in altered proximal insulin signaling, suggesting that the cellular regulatory responses to acute versus more chronically altered levels of MCoA may be mechanistically different. In this context, it is noteworthy that improved insulin-stimulated glucose uptake is observed in rodent muscle in recovery from exercise and electrical stimulation (35, 36) and in human skeletal muscle 4 h after acute exercise (37) without changes in...
proximal insulin signaling (52, 53). Furthermore, in rodent muscle a similar adaptive pattern is observed 3.5 h after acute pharmacological or hypoxia-induced AMPK activation (17). Whether improved insulin action in these situations is associated with altered cellular content of MCoA remains to be investigated.

In the present study, the effect of acute one-legged exercise on MCoA content and insulin-stimulated glucose uptake was evaluated 4 h after termination of exercise in human skeletal muscle. Furthermore, we investigated whether 4 days of diet designed to alter lipid availability and intramyocellular triacylglycerol (IMTG) content would alter the intracellular content of MCoA and insulin action.

**METHODS**

**Subjects.** Twelve young, healthy, moderately trained men [age 24 ± 1 yr; height 1.88 ± 0.02 m; body mass (BM) 81 ± 3 kg; body mass index 23.5 ± 0.5 kg/m²; peak oxygen uptake (VO₂peak)/BM during bicycling 55 ± 2 ml·kg⁻¹·min⁻¹] gave their informed consent to participate in the study, which was approved by the Copenhagen Ethics Committee (no. 01-180/01) and was in accordance with the Declaration of Helsinki II. The subjects had a <5% leg difference in estimated quadriceps femoris muscle mass. The results presented in this study are part of a larger study on the relation between diet, exercise, and insulin signaling in human skeletal muscle, which has been published previously in minor part (18).

**Study design.** Subjects underwent two experimental trials separated by 2–3 wk. In both trials subjects followed a controlled diet during the 4 days preceding the experiment (between day −4 and day 0, Fig. 1). In one trial the diet had a high fat content (Fat) and in the other trial the diet had a high carbohydrate content (CHO) (see Diets). On the experimental day subjects performed one-legged knee extension exercise for 1 h, and then 4 h later they underwent a 100-min euglycemic-hyperinsulinemic clamp (Fig. 1). The order of diets and whether exercise was performed with the dominant or nondominant leg were randomized.

**Preexperimental testing.** All subjects initially performed an incremental exercise test on a bicycle ergometer (Monark 839 Electronic Ergometer, Monark Exercise) to determine their VO₂peak. Respiratory measurements were carried out with the Douglas bag technique. Quadriceps femoris muscle mass was estimated in both legs as described previously (1) under the assumption that quadriceps femoris constitutes 40% of total thigh muscle mass (7).

**Diet.** For 4 days preceding each experiment, subjects consumed a diet containing 20 energy percent (E%) carbohydrate, 65 E% fat (primarily unsaturated), and 15 E% protein in the Fat trial and 65 E% carbohydrate, 20 E% fat, and 15 E% protein in the CHO trial. The constituents of the diet were weighed out at the laboratory and delivered to the subjects. The amount of energy to be consumed was individually determined from body weight and reported habitual physical activity level based on guidelines from the World Health Organization (2).

**Experimental protocol.** Subjects arrived at the laboratory in the morning after an overnight fast. They had a light, carbohydrate-rich breakfast (5% of daily energy intake) and then completed an exercise bout consisting of 60-min dynamic one-legged knee extensor exercise (1 kick/s) at ~80% of the knee extensors’ peak workload (PWL) with 2 × 5-min periods at 100% PWL placed after 20 and 40 min in order to ensure activation of the majority of muscle fibers in the thigh region. The contralateral leg was kept at rest. After exercise subjects rested in the supine position, and Teflon catheters were inserted below the inguinal ligament in one femoral artery and both femoral veins. A thermodilution catheter (EdsLab probe 94-030-2.5F, Baxter, Allerod, Denmark) was inserted through both femoral venous catheters and advanced 8 cm proximally for determination of femoral venous blood flow. Two venous catheters were inserted into antecubital veins for infusion of insulin and glucose, respectively. Four hours after exercise, subjects underwent a 100-min euglycemic-hyperinsulinemic clamp (1.5 mU·min⁻¹·kg⁻¹ BM⁻¹) that was initiated with a bolus injection of insulin (9 mU/kg BM) (Actrapid, Novo Nordisk, Bagsvaerd, Denmark) administered over 1 min. Blood samples were drawn simultaneously from the femoral catheters before and during (15, 30, 45, 60, 80, 100 min) insulin infusion. Each blood withdrawal was followed by determination of femoral venous blood flow by the constant infusion thermodilution technique (37) after occlusion of the lower leg with an inflated cuff (>220 mmHg). This allowed for calculation of thigh glucose uptake with arteriovenous differences. During the clamp arterial blood samples were taken frequently and analyzed rapidly for glucose in order to adjust the glucose infusion rate. Muscle biopsies were obtained from the vastus lateralis muscle, in both the rested (Con) and the previously exercised (Exe) leg, before initiation and after 100 min of the clamp. Biopsies in the same leg were obtained from individual incisions separated by 5–6 cm. Before each muscle biopsy time point, expired air was collected in a Douglas bag over a 7-min period for determination of respiratory exchange ratio (RER).

**Breath samples.** Expired volumes of air in the Douglas bags were measured with a chain-suspended Collins spirometer, and a small sample of mixed expiratory air was analyzed for O₂ (Servomex S-3A) and CO₂ (Beckman LB2). RER was calculated as the ratio between pulmonary CO₂ excretion and O₂ uptake.

**Blood samples.** Blood glucose concentration was measured automatically (ABL510; Radiometer Medical, Copenhagen, Denmark). Plasma fatty acid (FA) concentration was measured by a colorimetric commercial assay kit (Wako Chemicals Richmond, VA) with a COBAS FARA autoanalyzer (COBAS FARA 2, Roche Diagnostic Rotkreuz, Switzerland). Concentrations of insulin (Pharmacia Insulin Radioimmunoassay 100; Pharmacia & Upjohn Diagnostics, Uppsala, Sweden) as well as epinephrine and norepinephrine (KatCombi Radioimmunoassay; Immuno-Biological Laboratories, Hamburg, Germany) in plasma were determined by radioimmunoassay.

**Muscle biopsies.** Biopsies were quick-frozen (<20 s) in liquid nitrogen and stored at −80°C for subsequent analysis. Wet muscle tissue was freeze-dried and dissected free of all visible adipose tissue, connective tissue, and blood.

**Intramyocellular triacylglycerol.** The concentration of IMTG was determined in freeze-dried and dissected muscle tissue as described previously (25). The concentration of IMTG was determined in freeze-dried and dissected muscle tissue by a fluorometric method (27).

**Malonyl-CoA.** The muscle MCoA concentration was measured by a method described previously (30) modified for human muscle tissue (13, 38). In short, 25 mg of muscle tissue (wt wt) was homogenized in 250 μl of perchloric acid (10%) and neutralized with NaOH. MCoA was determined in the neutralized muscle homogenates by measuring MCoA-dependent incorporation of [1H1]acetyl-CoA into palmitate
catalyzed by fatty acid synthase (kindly donated by Dr. J. Knudsen, University of Southern Denmark, Odense).

Muscle lysates. Freeze-dried and dissected muscle tissue was homogenized (1:80 wt/vol) in ice-cold buffer containing 50 mM HEPES (pH 7.5), 150 mM NaCl, 20 mM sodium pyrophosphate, 20 mM β-glycerophosphate, 10 mM NaF, 2 mM sodium orthovanadate, 2 mM EDTA, 1% NP-40, 10% glycerol, 2 mM PMSF, 1 mM MgCl₂, 1 mM CaCl₂, 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 3 mM benzamidine. Homogenates were rotated end over end for 1 h at 4°C and then cleared by centrifugation at 17,500 g at 4°C for 30 min. Protein content in the supernatant was measured by the bicinchoninic acid method (Pierce Rockford, IL).

Western blotting. Phosphorylation of AMPK Thr²⁷², ACC-β Ser²³⁷, Akt Ser⁴⁷³, Akt Thr³⁰⁸, GSK-3β Ser⁹, and glycogen synthase (GS) Ser⁷⁹ (site 3a) as well as GLUT4 and ACC-β protein content were detected by Western blotting on muscle lysates. Fifteen to sixty micrograms of lysate protein was boiled in Laemmli protein content were detected by Western blotting on muscle lysates. Fifteen to sixty micrograms of lysate protein was boiled in Laemmli buffer before being subjected to SDS-PAGE and immunoblotting. Rabbit anti-α-AMPK Thr²⁷²-phos, anti-Akt Ser⁴⁷³-phos, anti-GSK-3β Ser⁹-phos, and anti-GS Ser⁷⁹-phos antibodies were from Cell Signaling Technology (Beverly, MA). Rabbit anti-ACC-α Ser⁷⁹-phos and anti-Akt Thr³⁰⁸-phos were from Upstate Biotechnology (Lake Placid, NY). Rabbit anti-GLUT4 was from Affinity BioReagents (Golden, CO). Horseradish peroxidase-conjugated anti-rabbit (DAKO, Glostrup, Denmark) was used as secondary antibody. ACC-β contains a biotin moiety that is recognized by streptavidin, and therefore horseradish peroxidase-conjugated streptavidin (DAKO) was used to detect ACC-β protein. Anti-antibody complexes were visualized with enhanced chemiluminescence (ECL+., Amersham Biosciences) and quantified by a Kodak Image Station 2000MM (Kodak, Glostrup, Denmark). Representative blots are shown in Fig. 2.

Insulin receptor substrate-1-associated phosphatidylinositol 3-kinase activity. Insulin receptor substrate (IRS)-1 was immunoprecipitated from muscle homogenates (400 μg lysate protein) with an anti-IRS-1 antibody (Upstate Biotechnology). Phosphatidylinositol 3-kinase (PI3-kinase) activity in the IRS-1 immunoprecipitates was determined as described previously (53). Briefly, PI3-kinase activity was assayed for 30 min, allowing for ³²P incorporation into phosphatidylinositol (PI). Subsequently, PI was isolated with thin-layer chromatography and the amount of radioactivity incorporated was detected by phosphorimaging (Storm 840, Molecular Dynamics, Amersham Pharmacia Biotech, Little Chalfont, UK) and expressed in arbitrary units relative to a standard.

AMPK complex activities. Activity of the three AMPK complexes (α1β2y1, α₂βγ2y1, and α₂ββγ2y3) expressed in human muscle was measured in vitro in muscle lysates (200 μg) after sequential immunoprecipitation of each complex as previously described in detail (6).

Statistics. Data are presented as means ± SE. For variables measured after both diets either before and during the euglycemic-hyperinsulinemic clamp or in Con and Exe legs before the clamp, a two-way analysis of variance with repeated measures was performed to test for differences between factors. For variables that were measured after both diets in Con and Exe legs both before and during the clamp, a three-way analysis of variance with repeated measures was performed to test for differences between factors. When significant main effects were found or when significant interaction between effects of diet, leg, and/or time was found, pairwise differences were tested with a Tukey post hoc test. Correlation analysis was performed by a Pearson product moment test. In all cases, a probability of 0.05 was used as the level of significance.

RESULTS

Respiratory exchange ratio. Basal RER did not differ significantly between Fat and CHO diets, and an increase (P < 0.001) was observed in response to insulin in both trials (Table 1).

Femoral venous blood flow. Femoral venous blood flow was similar in the two thighs at rest and during the clamp and was not influenced by the preceding diet [not significant (NS)]. Blood flow increased (P < 0.001) in response to insulin stimulation (Fat,Con: 439 ± 49; Fat,Exe: 453 ± 44; CHO,Con: 471 ± 37; CHO,Exe: 514 ± 42 ml/min) compared with basal levels (Fat,Con: 409 ± 61; Fat,Exe: 410 ± 57; CHO,Con: 371 ± 31; CHO,Exe: 411 ± 44 ml/min).

Glucose kinetics. The glucose infusion rate during the euglycemic-hyperinsulinemic clamp did not differ significantly between Fat and CHO diets (NS) (Table 1). Arterial glucose was clamped at 5.2 ± 0.2 mM after both trials (Table 1). Data on thigh glucose uptake after the CHO diet (but not the Fat diet) are constructed from previously published time course curves of glucose uptake (18). Thigh glucose uptake increased (P < 0.001) in response to insulin stimulation in both legs but, however, to a significantly greater extent (P < 0.001) in the Exe leg (Fig. 3). Furthermore, a tendency (P = 0.057) toward generally lower thigh glucose uptake was observed after the Fat diet compared with the CHO diet (Fig. 3).

Plasma FA. The basal arterial plasma FA concentration did not differ significantly between Fat and CHO diets, and a decrease (P < 0.01) was observed in response to insulin after both diets (Table 1).

Circulating hormones. At basal conditions the arterial concentrations of insulin, epinephrine, and norepinephrine in plasma were similar after Fat and CHO diets (NS). In response to insulin infusion concentrations increased (P < 0.01) to similar levels after the two diets (NS) (Table 1).

 Intramyocellular triacylglycerol. The IMTG concentration in the vastus lateralis muscle was ~40% higher (P < 0.01) after the Fat diet compared with the CHO diet (Fig. 4A). The IMTG concentration did not differ between Con and Exe legs (NS) and did not change with insulin stimulation (NS).
There was no effect of diet on MCoA content (NS).

Muscle glycogen. The glycogen concentration in the vastus lateralis muscle was ~40% higher ($P < 0.01$) after the CHO diet compared with the Fat diet (Fig. 4B). In the Exe leg, muscle glycogen content was reduced by ~30% ($P < 0.001$) compared with the Con leg. Furthermore, in the Exe leg glycogen content increased ~10% ($P < 0.01$) during the euglycemic-hyperinsulinemic clamp.

Malonyl-CoA. The MCoA content in the vastus lateralis muscle was ~20% lower ($P < 0.05$) in the Exe leg compared with the Con leg. A ~25% increase was observed in MCoA content during the euglycemic-hyperinsulinemic clamp (Fig. 5A). There was no effect of diet on MCoA content (NS).

Correlation analysis. We tested whether the difference in basal MCoA content between Con and Exe correlated with the difference between Exe and Con in thigh glucose uptake during the euglycemic-hyperinsulinemic clamp. It was found that these two parameters correlated after both the Fat diet ($r = 0.63, P < 0.05$) and the CHO diet ($r = 0.68, P < 0.05$). When Fat and CHO diets were combined, the correlation coefficient was similar ($r = 0.65, P < 0.001$) (Fig. 5B).

AMPK and ACC. Muscle AMPK Thr$^{172}$ phosphorylation and in vitro activity of AMPK complexes did not differ between Fat and CHO diets or between Con and Exe legs (NS) (Figs. 6A and 7). Muscle ACC-$\beta$ Ser$^{217}$ phosphorylation was ~20% higher ($P < 0.05$) 4 h after exercise before insulin stimulation and ~25% lower ($P < 0.05$) after the CHO diet compared with the Fat diet (Fig. 6B). This latter difference prompted us to measure ACC-$\beta$ protein content (Fig. 6C). ACC-$\beta$ protein content in muscle was ~20% lower after the CHO diet compared with the Fat diet. Thus when ACC-$\beta$ Ser$^{217}$ phosphorylation was expressed per ACC-$\beta$ protein no significant difference between CHO and Fat diets was observed; however, the exercise effect was maintained (data not shown).

PI3-kinase activity and Akt phosphorylation. Since data from the CHO diet have been published previously (18), only data from the Fat trial are illustrated (Fig. 8). Comparisons between diets are made with reference to the previously published data. Basal IRS-1-associated PI3-kinase activity in the vastus lateralis muscle did not differ between Fat and CHO diets or between Con and Exe muscle (NS). IRS-1-associated PI3-kinase activity increased ~150% ($P < 0.001$) during the euglycemic-hyperinsulinemic clamp after both diets. However,
insulin-stimulated IRS-1-associated PI3-kinase activity after the Fat diet was \(20\%\) lower in the Con leg compared with the Exe leg \((P < 0.01)\) and compared with both legs after the CHO diet \((P < 0.01)\) (Fig. 8A; Ref. 18).

Akt Thr\(^{308}\) and Ser\(^{473}\) phosphorylation increased \(400\%\) \((P < 0.001)\) in response to insulin stimulation after both diets (Fig. 8B and C). However, insulin-stimulated Akt Thr\(^{308}\) phosphorylation was \(15\%\) lower \((P < 0.01)\) in both legs after the Fat diet compared with the CHO diet (Fig. 8B; Ref. 18).

**GSK-3 and GS phosphorylation.** Basal GSK-3\(\alpha\) Ser\(^{21}\) and GSK-3\(\beta\) Ser\(^{9}\) phosphorylation did not differ between the Fat and CHO diets or between Con and Exe muscles. In response to insulin stimulation, phosphorylation (leading to inhibition) on both proteins increased \(100\%\) \((P < 0.05)\) (Fig. 9A and B). GS Ser\(^{640}\) phosphorylation (leading to inhibition) was decreased \(30\%\) \((P < 0.001)\) in Exe muscles compared with Con muscles after both diets (Fig. 9C). No significant effect of diet \((P = 0.13)\) was observed. In response to insulin stimulation, GS Ser\(^{640}\) phosphorylation decreased \(50\%\) \((P < 0.001)\) in both legs independent of diet.

**GLUT4 protein content.** Content of GLUT4 did not differ between Exe and Con muscle or change in response to diet or insulin stimulation (Fig. 9D).

A novel finding in the present study was the observation of a reduction in content of MCoA 4 h after acute exercise in human skeletal muscle. Interestingly, the reduction significantly correlated \((r = 0.65, P < 0.001)\) with improved insulin-stimulated thigh glucose uptake (Fig. 5B). Furthermore, a diet-induced \(~40\%\) increase in IMTG content in skeletal muscle was observed.

**DISCUSSION**

A: malonyl-CoA concentration in the vastus lateralis muscle before and after a 100-min euglycemic-hyperinsulinemic clamp following Fat or CHO diet and acute 1-legged exercise. *Main effect of insulin, \(P < 0.01\); #main effect of exercise, \(P < 0.05\). Data are means ± SE; \(n = 12\). w.w., Wet weight.

B: relationship between the effect of prior exercise on muscle malonyl-CoA content and on insulin-stimulated thigh glucose uptake.

**Fig. 5.** A: malonyl-CoA concentration in the vastus lateralis muscle before and after a 100-min euglycemic-hyperinsulinemic clamp following Fat or CHO diet and acute 1-legged exercise. *Main effect of insulin, \(P < 0.01\); #main effect of exercise, \(P < 0.05\). Data are means ± SE; \(n = 12\). w.w., Wet weight. B: relationship between the effect of prior exercise on muscle malonyl-CoA content and on insulin-stimulated thigh glucose uptake.

**Fig. 6.** AMPK Thr\(^{172}\) phosphorylation (A), ACC-\(\beta\) Ser\(^{21}\) phosphorylation (B), and ACC-\(\beta\) protein content in the vastus lateralis muscle (C) before and after a 100-min euglycemic-hyperinsulinemic clamp following Fat or CHO diet and acute 1-legged exercise. AU, arbitrary units. †Main effect of diet, \(P < 0.05\); #effect of exercise at basal level, \(P < 0.05\). Data are means ± SE; \(n = 12\).
muscle was not reflected in decreased whole body insulin action, whereas insulin action of thigh glucose uptake was marginally ($P = 0.057$) decreased compared to a carbohydrate-rich diet (Fig. 3).

As supported by the present measurements of IRS-1-associated PI3-kinase activity, Akt phosphorylation (Fig. 8) and GLUT4 protein content (Fig. 9D) and, as previously reported (18, 52, 53), improved insulin-stimulated glucose uptake 4 h after exercise cannot be ascribed to improved proximal insulin signaling or increased total content of GLUT4. Consistently, we also show that downstream of Akt, insulin-stimulated phosphorylation of GSK-3α/β (leading to deactivation) and subsequent dephosphorylation of GS (site 3a) is not more potently regulated after exercise (Fig. 9, A–C). Together with the reduced MCoA content following exercise in the present study, these findings are in accordance with observations in myotubes suggesting that acute alterations of MCoA content in muscle cells may inversely influence insulin-stimulated glucose uptake independent of proximal insulin signal interactions (32). It should be emphasized that despite normal insulin action on IRS-1-associated PI3-kinase activity after exercise, we (18) and others (23) have previously shown that insulin-stimulated PI3-kinase activity associated with IRS-2 is greater after exercise. Interestingly, the most pronounced effect of exercise is observed immediately after termination of exercise at a time point when whole body insulin action is not improved (23). Furthermore, 4 h after exercise, increased IRS-2-associated PI3-kinase activity is observed also in response to basal insulin concentrations despite an unaltered basal glucose uptake (18). These observations seem to dissociate IRS-2 signaling from regulation of glucose uptake in humans, in accordance with results obtained from IRS-2-deficient mice (21) and human skeletal muscle myotubes after small interfering RNA (siRNA) silencing of IRS-2 (10).

The mechanism to explain the association between acutely altered MCoA levels and insulin action is presently unknown; however, it is reasonable to assume that it relates to the role of MCoA in regulation of lipid oxidation. Thus, similar to the lipid intermediary long-chain acyl-CoA (LCACoA) has been shown to inhibit hexokinase activity in homogenates from rat and human skeletal muscle in vitro (46). In light of results from conscious unrestrained mice, the capacity of hexokinase to phosphorylate glucose may present a barrier for glucose uptake in response to hyperinsulinemia (19). Thus reduced content of LCACoA in recovery from exercise could provide a mechanism whereby insulin-stimulated glucose uptake is increased independently of insulin signaling capacity.

It can be speculated that reduced MCoA content in recovery from exercise contributes to the content of yet undefined lipid species in skeletal muscle with direct impact on glucose uptake and metabolism in muscle, independent of interactions with insulin signaling. Reduced cellular lipid content during recovery from exercise seems a plausible scenario given the previous observation of a continued increase in lipid oxidation at least 3 h into recovery from one-legged exercise (2 h, 65% PWL) (48) despite a rapid reversal (to basal levels) of muscle fatty acid uptake after termination of exercise (48, 55). Furthermore, in accordance with previous observations (24), we do not observe a detectable reduction in MCoA content after one-legged exercise (Fig. 4), suggesting a negligible contribution of IMTG to lipid oxidation during and in recovery from exercise with a low systemic impact, as opposed to our previous observation in recovery from exhaustive whole body exercise (26). We hypothesize that the present observation of a continued reduction in MCoA content 4 h after exercise may be part of the adaptive response explaining increased lipid oxidation in recovery from exercise. In fact, the role of MCoA to stimulate lipid oxidation may be particularly evident after exercise since the inhibitory effect of MCoA on CPT I activity is reduced in response to exercise when evaluated in vitro (22).

Interestingly, improved insulin action after exercise correlated with the reduced MCoA content at initiation of the clamp but not at termination of the clamp (data not shown). This would be consistent with an indirect role of MCoA in regulating insulin action and suggests a certain time delay between alterations in MCoA content and altered insulin action in vivo. Future studies are needed to confirm causality of the present correlational observations; furthermore, candidate effector molecules need to be identified. In this context it is noteworthy that the lipid intermediary long-chain acyl-CoA (LCACoA) has been shown to inhibit hexokinase activity in homogenates from rat and human skeletal muscle in vitro (46). In light of results from conscious unrestrained mice, the capacity of hexokinase to phosphorylate glucose may present a barrier for glucose uptake in response to hyperinsulinemia (19). Thus reduced content of LCACoA in recovery from exercise could provide a mechanism whereby insulin-stimulated glucose uptake is increased independently of insulin signaling capacity.

Fig. 7. AMPK activity associated with the 3 human AMPK complexes ($\alpha$B2y1, $\alpha$B2y1, and $\alpha$B2y3) before the insulin clamp in vastus lateralis muscle following Fat or CHO diet. Data are means ± SE; $n = 12$. AJP-Endocrinol Metab · VOL 296 · APRIL 2009 · www.ajpendo.org

Fig. 8. Insulin receptor substrate (IRS)-1-associated phosphatidylinositol 3-kinase (PI3-kinase) activity, Akt Thr308 phosphorylation, and Akt Ser473 phosphorylation in the vastus lateralis muscle before and after a 100-min euglycemic-hyperinsulinemic clamp following Fat diet and acute 1-legged exercise. A: IRS-1-associated PI3-kinase activity. *Main effect of insulin, $P < 0.001$; †different from EXE on Fat ($P < 0.01$); ‡values significantly lower than values obtained after CHO diet and published in Ref. 18, $P < 0.01$. B: Akt Thr308 phosphorylation. *Main effect of insulin, $P < 0.001$; ‡values significantly lower than values obtained after CHO diet and published in Ref. 18, $P < 0.01$. C: Akt Ser473 phosphorylation. *Main effect of insulin, $P < 0.001$. Data are means ± SE; $n = 12$. AJP-Endocrinol Metab · VOL 296 · APRIL 2009 · www.ajpendo.org
A range of adaptations are probably corequired in order to fully explain improved insulin-stimulated glucose uptake in recovery from exercise. Thus several previous observations are not easily associated with alterations in muscle content of MCoA. These include the following: 1) Improved insulin action after exercise is associated with increased insulin-stimulated translocation of GLUT4 to the plasma membrane in rodent muscle (20). 2) Improved insulin action after exercise is correlated with the reduction in glycogen content during exercise (54). 3) Improved insulin action after exercise is associated with altered function of distal insulin signaling molecules, including atypical PKC (aPKC) and AS160 (3, 18). However, the present findings inspire a closer inspection of skeletal muscle lipid status in recovery from exercise in order to more completely understand the cellular events leading to improved insulin action after exercise. In regard to signaling downstream of Akt to AS160, we have recently discovered that AS160 is hyperphosphorylated on four sites (Ser318, Ser341, Ser588, and Ser751) observable in both basal (around 40%) and insulin-stimulated (around 20%) muscle 4 h after exercise in humans. In contrast, neither insulin-stimulated phosphorylation of AS160 Ser642 nor AS160 phosphorylation detected with the phospho-Akt substrate (PAS) antibody is altered (47a). Enhanced AS160 phosphorylation in response to insulin stimulation after contractions has previously been reported with the PAS antibody in rodents (3); thus despite species differences our human data confirm that AS160 may be a point of convergence between exercise and insulin signaling to glucose uptake.

As described, acute alteration in MCoA content is considered an important mechanism to regulate FA oxidation in rodents. Accordingly, increased lipid oxidation during and in recovery from contractions in rodent muscle is associated with reduced MCoA content (34, 49). This may in part result from acute and sustained AMPK-mediated inactivation of ACC-β and activation of MCD (14, 34, 42, 49). Whether this series of events is also involved in acute exercise-induced stimulation of FA oxidation in human muscle is less clear. Thus the concentration of MCoA is 1/10 in human muscle compared with rodents (13), and in humans the exercise-induced decrease in content of MCoA is of a minor magnitude (10–20% of resting values) compared with rodent observations (50% of resting values) (13, 31, 38, 49). Furthermore, despite similar exercise-induced reductions in MCoA content in humans, muscle with low preexercise glycogen content has markedly elevated fat oxidation during exercise compared with muscle with high glycogen content (38). This suggests only a minor or partial role of MCoA in acute regulation of fat oxidation during exercise in humans. In contrast, siRNA silencing of the MCD gene expression in cultured myotubes from resting human muscle leads to increased MCoA content and subsequently markedly reduced lipid oxidation (9), supporting a direct inhibitory role of MCoA on lipid oxidation at rest. In the present study, content of MCoA was reduced by 30% 4 h after termination of acute exercise (Fig. 5A), independent of preexercise diet composition. This was associated with a sustained increase in ACC-β Ser21 phosphorylation (Fig. 6B), suggesting a continued suppression of MCoA synthesis in recovery.
from exercise. In contrast, upstream activity of AMPK was reversed to resting values at this time point, as evaluated by total AMPK Thr172 phosphorylation (Fig. 6A) as well as in vitro activity measurements of the three AMPK complexes (α1β2γ1, α2β2γ1, and α2β2γ3) existing in human muscle (Fig. 7; Ref. 6). This suggests a certain time delay in reversal of signaling downstream of AMPK after termination of exercise, although a potential impact of other ACC Ser253 kinases or phosphatases cannot be ruled out (16). Furthermore, compared with the rapid reversal (within 1 h of recovery) of both ACC-β Ser237 phosphorylation and AMPK activity associated with α2-containing complexes in response to 1 h of cycle exercise (70% of VO2peak) in very well-trained subjects (55), it appears that signaling downstream of AMPK in recovery from exercise is influenced by exercise intensity as well as training status in humans.

In response to 4 days of high-fat diet primarily consisting of unsaturated fat, an ~40% increase in IMTG content in skeletal muscle was observed compared with an isocaloric high-carbohydrate diet (Fig. 4A). In contrast, the diet intervention did not significantly influence muscle MCoA content or whole body glucose infusion rate under hyperinsulinemia (Table 1). This latter finding is in agreement with previous observations of normal (or even slightly increased) insulin-stimulated glucose uptake at the whole body level after isocaloric high-fat diets for 6–21 days (8, 11, 12). In contrast, after 3 days of isocaloric high-fat diet, impaired insulin action has been detected at the whole body level in response to an oral glucose tolerance test (33). The reason for these discrepancies likely relates to different dietary composition and methods of evaluation; however, it cannot be ruled out that compensatory adaptations occur in response to more prolonged high-fat diet interventions rescuing whole body insulin action (12).

Contrary to whole body insulin action, insulin-stimulated glucose uptake in muscle tended to be lower (P = 0.057) when evaluated in both thighs after the fat-rich diet (Fig. 3). To our knowledge this is the first study to investigate insulin action on glucose uptake in muscle in response to a high-fat diet compared with an isocaloric high-carbohydrate diet. In the present investigation reduced insulin-stimulated glucose uptake coincided with impaired insulin-stimulated IRS-1-associated PI3-kinase activity and Akt Thr308 (but not Akt Ser473) phosphorylation. It should be emphasized that interactions with insulin signaling were of a minor magnitude and were not consistently observed in the previously exercised muscle. In the view of the authors, a reasonable interpretation is that even under these extreme diet conditions interference with insulin signaling to glucose uptake is minor and at the limit of analytical detection. Furthermore, it should be emphasized that under the present experimental conditions impairment of proximal insulin signaling was not associated with detectable changes in muscular content of MCoA (Fig. 5A).

In summary, this study shows that human skeletal muscle MCoA content is reduced 4 h after termination of acute one-legged exercise and that the reduction in MCoA content correlated with improved insulin-stimulated carbohydrate uptake at this time point. Since systemic impact during this type of exercise is marginal, these adaptations should primarily be ascribed to local contraction-induced mechanisms. Four days of high-fat diet resulted in an increased content of IMTG but did not influence muscle MCoA content or whole body insulin-stimulated glucose uptake compared with an isocaloric high-carbohydrate diet. However, at the muscular level proximal insulin signaling and insulin-stimulated glucose uptake appeared to be compromised although to a minor extent by the high-fat diet.

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