Am J Physiol Endocrinol Metab 292: E1740–E1749, 2007.
First published January 30, 2007; doi:10.1152/ajpendo.00579.2006.

A null mutation in skeletal muscle FAT/CD36 reveals its essential role in insulin- and AICAR-stimulated fatty acid metabolism

**Arend Bonen,[1] Xiao-Xia Han,[1] Daphna D. J. Habets,[2]
Maria Febbraio,[3] Jan F. C. Glatz,[2] and Joost J. F. P. Luiken[2,4]**

[1]*Department of Human Health and Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada;* [2]*Department of Molecular Genetics, Maastricht University, Maastricht, The Netherlands;* [3]*Department of Cell Biology, Lerner Research Institute, Cleveland Clinic Cleveland, Ohio; and* [4]*Department of Biochemical Physiology and Institute of Biomembranes, Utrecht University, Utrecht, The Netherlands*

Submitted 31 October 2006; accepted in final form 12 January 2007

Bonen A, Han X-X, Habets DD, Febbraio M, Glatz JF, Luiken JJ. A null mutation in skeletal muscle FAT/CD36 reveals its essential role in insulin- and AICAR-stimulated fatty acid metabolism. *Am J Physiol Endocrinol Metab* 292: E1740–E1749, 2007. First published January 30, 2007; doi:10.1152/ajpendo.00579.2006.—Fatty acid translocase (FAT)/CD36 is involved in regulating the uptake of long-chain fatty acids into muscle cells. However, the contribution of FAT/CD36 to fatty acid metabolism remains unknown. We examined the role of FAT/CD36 on fatty acid metabolism in perfused muscles (soleus and red and white gastrocnemius) of wild-type (WT) and FAT/CD36 null (KO) mice. In general, in muscles of KO mice, *1*) insulin sensitivity and 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) sensitivity were normal, *2*) key enzymes involved in fatty acid oxidation were altered minimally or not at all, and *3*) except for an increase in soleus muscle FATP1 and FATP4, these fatty acid transporters were not altered in red and white gastrocnemius muscles, whereas plasma membrane-bound fatty acid binding protein was not altered in any muscle. In KO muscles perfused under basal conditions (i.e., no insulin, no AICAR), rates of hindquarter fatty acid oxidation were reduced by 26%. Similarly, in oxidative but not glycolytic muscles, the basal rates of triacylglycerol esterification were reduced by 40%. When muscles were perfused with insulin, the net increase in fatty acid esterification was threefold greater in the oxidative muscles of WT mice compared with the oxidative muscles in KO mice. With AICAR-stimulation, the net increase in fatty acid oxidation by hindquarter muscles was 3.7-fold greater in WT compared with KO mice. In conclusion, the present studies demonstrate that FAT/CD36 has a critical role in regulating fatty acid esterification and oxidation, particularly during stimulation with insulin or AICAR.

perfusion; palmitate; esterification; oxidation; fatty transport proteins 1 and 2; plasma membrane-bound fatty acid binding protein; 5-amino-imidazole-4-carboxamide-1-β-D-ribofuranoside

IN RECENT YEARS it has been established that fatty acid transport into metabolically active tissues such as heart and skeletal muscle occurs, in part, via a protein-mediated process (4, 7–9, 32, 33). Although a number of fatty acid transporters have been identified, it appears that fatty acid translocase (FAT)/CD36 and plasma membrane-bound fatty acid binding protein (FABPpm) are two key fatty acid transporters. Upregulation of FAT/CD36 and FABPpm, either after 7 days of chronic muscle contraction or after exposure of cardiac myocytes to insulin or 5-amino-imidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR), increases the rates of fatty acid transport (1, 5, 11, 13, 27). Conversely, when FAT/CD36 and FABPpm are concurrently downregulated, the rates of fatty acid transport into resting muscle are decreased (27, 44). The content of plasmalemmal FAT/CD36, but not FABPpm, is increased in insulin-resistant skeletal muscle, and this correlates with intramuscular triacylglycerol accumulation (9, 31). Despite these associations of FAT/CD36 and FABPpm with fatty acid transport and metabolism, the relative importance of these two fatty acid transporters is not known. Indirect evidence suggests that FAT/CD36 may be the more critical fatty acid transporter, given that the relative increase in plasmalemmal FABPpm, when overexpressed in vivo, did not scale well with the increased rate of fatty acid transport (14), and independent overexpression of FAT/CD36 and FABPpm suggested that FAT/CD36 has a greater capacity to upregulate fatty acid transport than FABPpm (36). Collectively, these studies suggest that FAT/CD36 has a key role in fatty acid metabolism.

Whether fatty acid metabolism is largely determined by the altered activity/capacity of selected enzymes involved in fatty acid esterification and/or oxidation or by the FAT/CD36-mediated influx of fatty acids is unclear. Based on studies conducted under basal conditions in skeletal muscle of FAT/CD36 null and overexpressing mice, there is conflicting evidence as to whether this transporter is important in regulating fatty acid metabolism (15, 26). For example, incorporation of iodinated fatty acid analogs into triacylglycerols was reduced in heart, skeletal muscle, and adipocytes of FAT/CD36 null mice (15). However, the converse was not observed when FAT/CD36 was overexpressed, since basal rates of skeletal muscle fatty acid oxidation and esterification were not altered despite a two- to fourfold increase in FAT/CD36 (26). These studies appear to suggest that FAT/CD36-mediated rates of fatty acid transport may only have limited effects on basal rates of fatty acid metabolism. However, when FAT/CD36-overexpressing muscles were stimulated to contract, it appeared that the resulting upregulation of fatty acid oxidation was closely linked to the increased FAT/CD36-mediated flux (26). Subsequent studies have shown that FAT/CD36 may be induced to translocate from an endosomal pool to the plasma membrane within minutes by muscle contraction (7, 46), by insulin (12, 33, 34), and by AICAR (12, 32). Therefore, in previous studies (15), the role of FAT/CD36 in regulating fatty acid metabolism may

Address for reprint requests and other correspondence: A. Bonen, Dept. of Human Health and Nutritional Science, Univ. of Guelph, Guelph, Ontario, Canada N1G 2W1 (e-mail: abonen@uoguelph.ca).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

 0193-1849/07 $8.00 Copyright © 2007 the American Physiological Society

Downloaded from http://ajpendo.physiology.org/ by 10.220.33.3 on May 28, 2017

have been underestimated when examined only under basal conditions and not during metabolic challenges. Along similar lines, the critical role of the glucose transporter GLUT4 in mediating glucose transport only became evident when GLUT4 null muscles were challenged with insulin or muscle contraction, since basal rates of glucose transport did not differ in wild-type and GLUT4 null mice (39, 45). Thus, to establish unequivocally the role of FAT/CD36 in fatty acid metabolism, it is necessary to examine fatty acid metabolism in FAT/CD36 null mice during selected metabolic challenges. This may also reveal whether fatty acid transport is rate limiting under different physiological conditions, as has been shown for GLUT4 (21), since FAT/CD36 can be induced to translocate to the plasma membrane by selected metabolic stimuli (7, 12, 32–34, 46).

In the present studies we have examined the rates of fatty acid esterification and oxidation in perfused hindlimb muscles of wild-type and FAT/CD36 null mice under basal conditions as well as during metabolic challenges with insulin and with AICAR. Each of these metabolic challenges normally induces the translocation of FAT/CD36 and thereby markedly increases the rates of fatty acid transport (7, 11, 12, 32–34). In addition, insulin stimulates skeletal muscle fatty acid esterification (19, 33), whereas AICAR stimulates fatty acid oxidation by activating AMP kinase (12, 32, 42). The present studies have shown that FAT/CD36 is critically important for supporting the increase in *1*) fatty acid esterification when challenged with insulin and *2*) fatty acid oxidation when challenged with AICAR.

METHODS

Materials

Antibodies against FAT/CD36, fatty acid transport proteins 1 and 4 (FATP1 and FATP4; Santa Cruz Biotechnology, Santa Cruz, CA), carnitine palmitoyltransferase 1 (CPT1; Cedarlane, Burlington, ON, Canada), cyclooxygenase-4 (COX-4; Invitrogen, Burlington, ON, Canada), AMP-activated protein kinase (AMPKα2; Upstate, Lake Placid, NY), phosphorylated acetyl-CoA carboxylase (ACC, Ser^{79}; Cell Signaling Technology, Danvers, MA), and phosphorylated AMPK (Thr^{172}; Upstate) were obtained from commercial sources. Anti-FABPpm was a gift from Dr. J. Calles-Escandon (Wake Forest University, Winston-Salem, NC). Bovine serum albumin and reagents for enzyme analyses were obtained from Sigma-Aldrich (St. Louis, MO). $[^{14}C]$palmitate and D-$[^{3}H]$glucose were obtained from Amersham Life Science (Little Chalfont, UK).

Animals

FAT/CD36 null mice were generated by targeted homologous recombination and crossed back six times to the C57Bl/6 background (20). Separate groups of wild-type (WT) and FAT/CD36 null (KO) mice were bred on site at the University of Guelph. Mice (males only, 21 ± 0.6 wk old; weight: WT, 36.6 ± 1.0 g and KO, 36.2 ± 1.4 g) were housed in a temperature (20°C)-regulated environment with a reversed 12:12-h light-dark cycle. Mice had access to standard laboratory chow and water ad libitum. Approval for all experiments was provided by the committee on animal care at the University of Guelph.

Hindlimb Perfusion

Palmitate esterification and oxidation were examined during *1*) basal (no insulin, no AICAR) conditions, *2*) insulin stimulation, and *3*) AICAR stimulation in perfused hindlimb muscles of fed WT and KO mice. These hindlimb perfusion procedures were similar to those our group has reported previously in rats (23, 29, 33), with only minor modifications to accommodate the much smaller mice. In addition, to validate the hindlimb perfusion procedure for mice, we conducted a preliminary experiment (n = 7). These pilot studies demonstrated that the perfused mouse hindlimb perfusion preparation was very stable, because *1*) ATP and ADP concentrations were not altered during the perfusion compared with freshly harvested muscle, and *2*) pH, P_{CO_2}, and P_{O_2} of the venous effluent were also unaltered (data not shown).

For the present studies, fed WT and KO mice were anesthetized with an intraperitoneal injection of pentobarbital sodium (65 mg/kg body wt) and surgically prepared for perfusion as has been done previously in rats (23, 29, 33). In all experiments, the hindquarters of mice were preperfused (20 min) and perfused (60 min) under identical conditions with a Krebs-Henseleit buffer (i.e., 95% O_2 and 5% of CO_2, pH 7.4, 37°C, 7 mM glucose, 0.5 mM palmitate, 4% bovine serum albumin, 3 ml/min flow rate, 100-ml recirculating volume). During the 20-min preperfusion period, the venous outflow was discarded. During the experiments (60 min), the perfusion medium (100 ml) was supplemented with 30 μCi, $[1\text{-}^{14}C]$palmitate, and 40 μCi D-$[^{3}H]$glucose. In three separate groups of WT and KO mice, muscles were perfused under basal conditions, with insulin (20 mU/ml), or with AICAR (2 mM). For the respective insulin and AICAR experiments, insulin (20 mU/ml) and AICAR (2 mM) were included during the preperfusion period. In all experiments (basal, insulin, and AICAR), arterial and venous perfusate samples were collected every 5 min for the last 30 min and kept in tightly sealed vials for later analysis of $^{14}CO_2$. After 60 min of perfusion, the experiments were stopped. Soleus and red and white gastrocnemius muscles were quickly harvested, rapidly frozen in liquid nitrogen, and stored at −80°C until analyzed for palmitate accumulation in the intramuscular phospholipids and di- and triacylglycerol depots.

CO_2 Production

Because fatty acid oxidation cannot be measured in individual hindlimb perfused muscles, the measurement of palmitate oxidation was performed across the perfused hindquarter, using procedures that our group has reported previously (33). Briefly, 0.5 ml of both arterial and venous perfusate samples were introduced into a tightly capped, sealed vial containing 1 M H_2SO_4 (1 ml). Liberated $^{14}CO_2$ was captured in benzethonium hydroxide and kept in a small Eppendorf tube securely centered in the vial. After 60 min, the tube containing benzethonium hydroxide with the released $^{14}CO_2$ was quickly transferred to a scintillation vial and counted for radioactivity. Standard calculations based on flow rate, timing of the sample, arteriovenous differences in $^{14}CO_2$, and specific activity of palmitate were used to calculate CO_2 production across the perfused hindquarter (33). Palmitate oxidation was adjusted for radioactive label fixation (18, 48). For these purposes, acid-soluble ^{14}C products in hindlimb muscles were obtained during the extraction of the lipids from the hindlimb muscles. This method has been widely used in isolated muscles (3, 5, 9, 18, 19, 37, 44) and in studies with fatty acid oxidation by isolated mitochondria (10, 28).

Palmitate Incorporation into Skeletal Muscle Phospholipids, Diacylglycerols, and Triacylglycerols

In soleus and red and white gastrocnemius muscles, palmitate incorporation into intramuscular phospholipids and tri- and diacylglycerols was determined using thin-layer chromatography as previously described in detail by our group (5, 18, 19, 33, 37). Rates of palmitate incorporation into the muscles were calculated based on the specific activity of the palmitate in the perfusion experiments.

Downloaded from http://ajpendo.physiology.org/ by 10.220.33.3 on May 28, 2017

Table 1. *Circulating concentrations of glucose, insulin, and fatty acids in wild-type and FAT/CD36 null mice*

Group	Glucose, mM	Insulin, pg/ml	Fatty Acids, mM
Wild type	7.5±0.3	190±20	0.52±0.04
FAT/CD36 null	8.0±0.4	220±30	0.70± 0.03*

Data are means ± SE; n = 7–10 mice. *P < 0.05, FAT/CD36 null vs. wild type.

Fatty Acid Uptake by Skeletal Muscle

It was unrealistic to determine rates of fatty acid transport into giant vesicles prepared from individual muscles, because that would have required pooling of individual muscles from ~30 mice for each independent experiment (i.e., ~1,080 mice in total for n = 6 per condition). Therefore, we have determined the rates of fatty acid uptake during the hindlimb perfusion. For these purposes, we calculated from the sum of palmitate esterfication (i.e., palmitate incorporation into phospholipids + di- + triacylglycerol) in each muscle. From this the total, palmitate esterification by the mouse hindquarter was calculated, taking into account the muscle mass and the fiber composition of mouse hindlimb muscles (Bonen A, unpublished data). This was then summed with the palmitate oxidized by the hindlimb muscles. Our group (29) has previously used a similar approach to determine glucose transport by the rat hindquarter based on data from individual muscles with different fiber types. In that study (29), the fold increments in glucose transport determined in the total hindlimb musculature corresponded closely with the fold increase in the insulin- and contraction-induced GLUT4 translocation in pooled hindlimb muscles.

Determination of Glycogen Synthesis

The rate of D-[^{3}H]glucose incorporation into muscle glycogen was based on procedures that Bonen and colleagues (6, 25) have previously used in isolated muscles. Briefly, frozen muscle samples were boiled in 1 N NaOH (0.3 ml) for 5 min and cooled on ice. A portion (10 μl) of the hydrolyzed muscle was used to determine protein concentration and the remainder was added in 0.5 ml of 30% KOH saturated with Na_2SO_4. Thereafter, the solution was boiled for another 5 min, and 0.5 ml of glycogen (40 mg/ml in 30% KOH) was added to act as a carrier . Glycogen was precipitated from muscle with 3 ml of 95% ethanol in H_2O at −20°C for 1 h. The precipitate was recovered with centrifugation at 4°C. The supernatant fraction was discarded, and the pellet was dissolved with 1 ml of H_2O and washed with 3 ml of 95% ethanol. Glycogen was pelleted with centrifugation at 4°C.

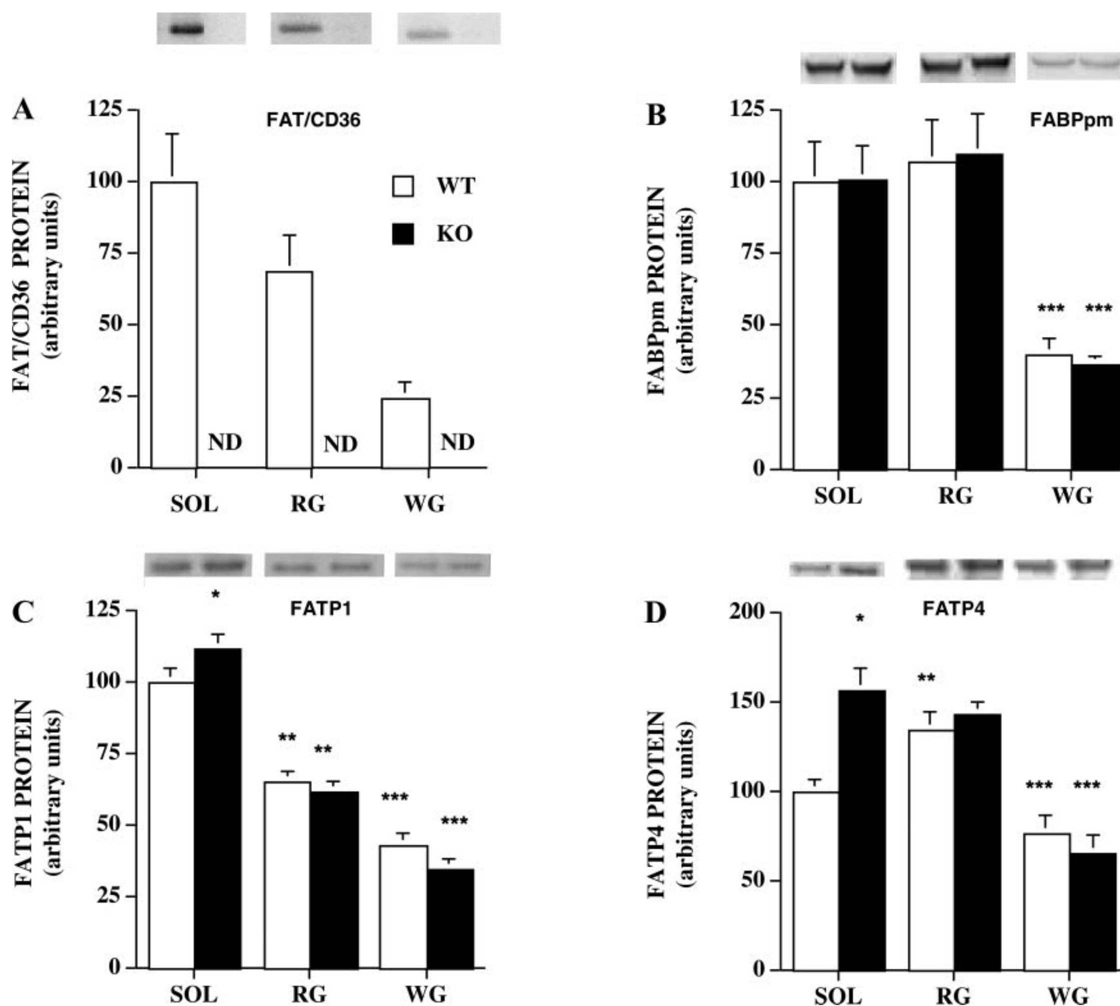

Fig. 1. Fatty acid transport proteins fatty acid translocase (FAT)/CD36 (*A*), plasma membrane-bound fatty acid binding protein (FABPpm; *B*), fatty acid transport protein (FATP)1 (*C*), and FATP4 (*D*) in soleus (SOL) and red (RG) and white gastrocnemius (WG) muscles of wild-type (WT) and FAT/CD36 null (KO) mice. Data are means ± SE; n = 8 for each muscle in each group. ND, not detected *P < 0.05, KO vs. WT muscle. **P < 0.05, RG vs. SOL in WT or KO mice. ***P < 0.05, WG vs. RG in WT or KO mice.

Downloaded from http://ajpendo.physiology.org/ by 10.220.33.3 on May 28, 2017

After washing (4×), the glycogen pellet was dissolved in 1 ml of H_2O, transferred to a scintillation vial, and counted for radioactivity.

Blood Sampling and Analyses

Blood samples were obtained from the inferior vena cava during the surgical preparation of the mice. Samples were analyzed for glucose (Sigma-Aldrich), insulin (Linco, St. Charles, MO), and fatty acids (Wako Chemicals, Richmond, VA) using commercially available procedures.

Enzyme Activities and Protein Expression

Muscles obtained from fed WT and KO mice were analyzed for fatty acid transporters (FAT/CD36, FABPpm, FATP1, and FATP4), CPT1, COX-4, AMPKα2, and phosphorylated ACC and AMPK with the use of standard preparations and Western blotting procedures as our group has previously reported (7, 10–12). The activities of β-hydroxyacyl-CoA dehydrogenase (β-HAD) and citrate synthase (CS) were also analyzed in muscle homogenates using well-known standard enzymatic procedures described by Lowry and Passonneau (30).

Statistics

The data were analyzed using repeated-measures analyses of variance (ANOVA), i.e., WT vs. KO mice, with repeated measures on muscles within each group. Post hoc tests were performed with the Fisher's least-squares difference procedure only when a significant F ratio was found in the ANOVA. Statistical significance was accepted at $P < 0.05$. All data are reported as means ± SE.

RESULTS

Glucose, Insulin, and Fatty Acids

In the fed WT and KO mice, there were no differences in circulating glucose or insulin concentrations ($P > 0.05$; Table 1). In contrast, circulating fatty acids were 35% higher in fed KO mice compared with fed WT mice ($P < 0.05$; Table 1). Others have found that glucose concentrations are lower in overnight-fasted KO mice compared with WT mice (22) and that circulating fatty acid concentrations were not different in fed KO and WT mice (15).

Comparisons among metabolically heterogeneous muscles in WT mice. In general, fatty acid transport proteins (Fig. 1), selected mitochondrial enzymes (Fig. 2), and insulin-stimulated rates of glycogenesis (Fig. 3) were greatest in the oxidative muscles (soleus and red gastrocnemius). Conversely, AMPKα2 protein and AICAR-stimulated AMPK phosphorylation were greatest in the fast-twitch red and white gastroc-

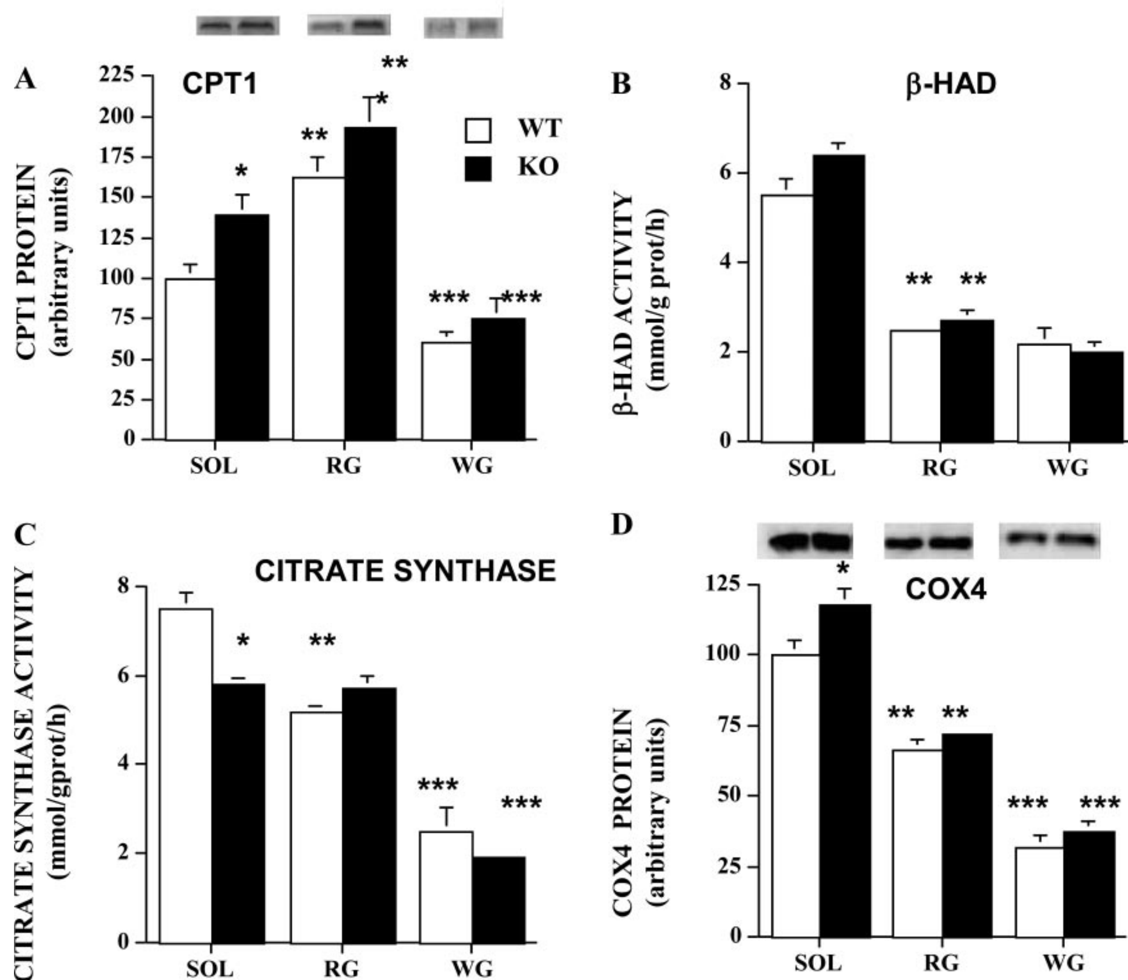

Fig. 2. Protein expression of carnitine palmitoyltranserase 1 (CPT1; *A*) and cyclooxygenase-4 (COX-4; *D*) and maximal activities of β-hydroxyacyl-CoA dehydrogenase (β-HAD; *B*) and citrate synthase (*C*) in SOL, RG, and WG muscles of WT and KO mice. Data are means ± SE; $n = 8$ for each muscle in each group. *$P < 0.05$, KO vs. WT. **$P < 0.05$, RG vs. SOL in WT or KO mice. ***$P < 0.05$, WG vs. RG in WT or KO mice.

Downloaded from http://ajpendo.physiology.org/ by 10.220.33.3 on May 28, 2017

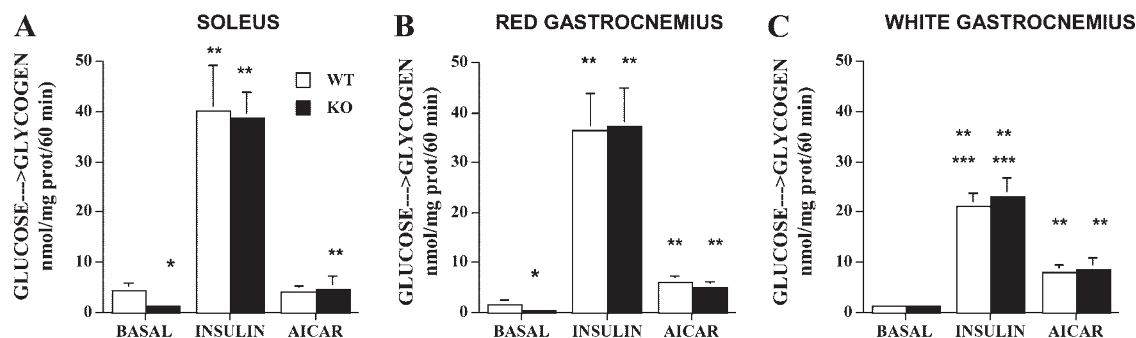

Fig. 3. Rates of glycogenesis under basal, insulin-stimulated, and 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR)-stimulated conditions in SOL (*A*), RG (*B*), and WG (*C*) muscles of WT and KO mice. Data are means ± SE; $n = 5$–7 for each muscle in each group. *$P < 0.05$, KO vs. WT. **$P < 0.05$, insulin- or AICAR-stimulated vs. basal. ***$P < 0.05$, WG vs. RG in WT or KO mice.

nemius muscles (Fig. 4, *A* and *B*), whereas AICAR-induced ACC phosphorylation was lower in these muscles (Fig. 4*C*).

With respect to fatty acid metabolism, rates of palmitate incorporation into phospholipids (Fig. 5, *A–C*) and diacylglycerols (Fig. 5, *D–F*) were greater in oxidative muscles (soleus and red gastrocnemius) than in white gastrocnemius muscle ($P < 0.05$). Phospholipid synthesis was not altered by insulin or AICAR stimulation in any muscle (Fig. 5, *A–C*), whereas insulin-mediated diacylglycerol formation was increased ($P < 0.05$) to a similar extent in all muscles (Fig. 5, *A–C*).

A large quantity of palmitate was incorporated into intramuscular triacylglycerols. In WT mice, basal rates of triacylglycerol formation in soleus muscle were 1.5- and 10-fold greater than in the red and white gastrocnemius muscles, respectively ($P < 0.05$; Fig. 5, *G–I*). Insulin stimulated the rate of palmitate incorporation into triacylglycerols in the soleus (+80%) and red gastrocnemius muscles (+59%) ($P < 0.05$; Fig. 5, *G* and *H*).

Comparisons between WT and KO mice. The different metabolic characteristics of the three muscles in WT mice were also generally observed among the muscles of KO mice. However, ablation of FAT/CD36 also resulted in marked differences in respective muscles of WT and KO mice.

Fatty Acid Transport and Mitochondrial Proteins

FAT/CD36 protein was not present in muscles of the KO mice. However, a compensatory increase occurred in FATP1 (+12%, Fig. 1*C*) and FATP4 (+57%, Fig. 1*D*) in soleus muscle only ($P < 0.05$). No differences in β-HAD activity were observed between respective muscles of KO and WT mice. However, in KO mice, CPT1 (soleus, +39%; red gastrocnemius, +20%; $P < 0.05$) and COX-4 (soleus, +18%) were increased ($P < 0.05$), whereas CS activity (soleus, −23%) was lower ($P < 0.05$).

Sensitivity to Insulin-Stimulated Glycogenesis

We assessed whether insulin sensitivity of glucose metabolism was maintained in muscles of FAT/CD36 null mice. In muscles of KO and WT mice, both the basal and the markedly increased insulin-stimulated rates of glycogenesis were similar (Fig. 3), and no differences were found in GLUT4 protein in muscles of WT and KO mice ($P > 0.05$; data not shown).

AMPKα2 and Effects of AICAR on Phosphorylation of AMPK and ACC

In KO soleus muscle, AMPKα2 protein was increased (+37%) compared with WT soleus muscle ($P < 0.05$; Fig. 4*A*), but the basal AMPK phosphorylation state was greater in red and white gastrocnemius of KO mice ($P < 0.05$; Fig. 4*B*). As in WT mice, AICAR also increased the phosphorylation of AMPK in red and white gastrocnemius muscles of KO mice ($P < 0.05$; Fig. 4*B*).

Under basal conditions ACC phosphorylation was increased in soleus (+117%) and white gastrocnemius muscles (+94%)

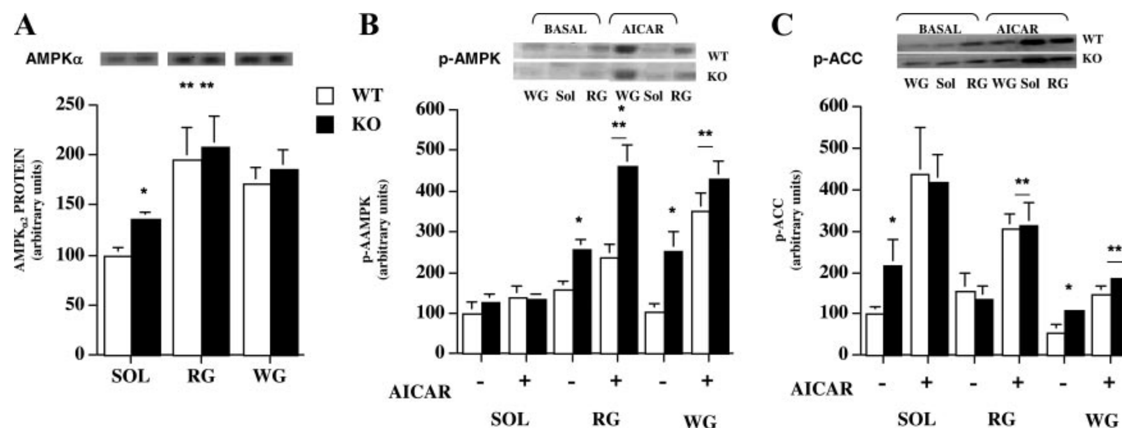

Fig. 4. Protein expression of AMP-activated protein kinase (AMPKα2; *A*) and AICAR-induced AMPK (*B*) and acetyl-CoA carboxylase (ACC) phosphorylation (*C*) in SOL, RG, and WG muscles of WT and KO mice. Data are means ± SE; $n = 8$ for each muscle in each group. *$P < 0.05$, KO vs. WT. **$P < 0.05$, RG or WG vs. SOL in WT or KO mice. ***$P < 0.05$, WG vs. RG in WT or KO mice. −$P < 0.05$, AICAR-induced phosphorylation vs. basal in either WT or KO mice. No differences in ACC phosphorylation were observed in respective muscles between WT and KO mice.

Downloaded from http://ajpendo.physiology.org/ by 10.220.33.3 on May 28, 2017

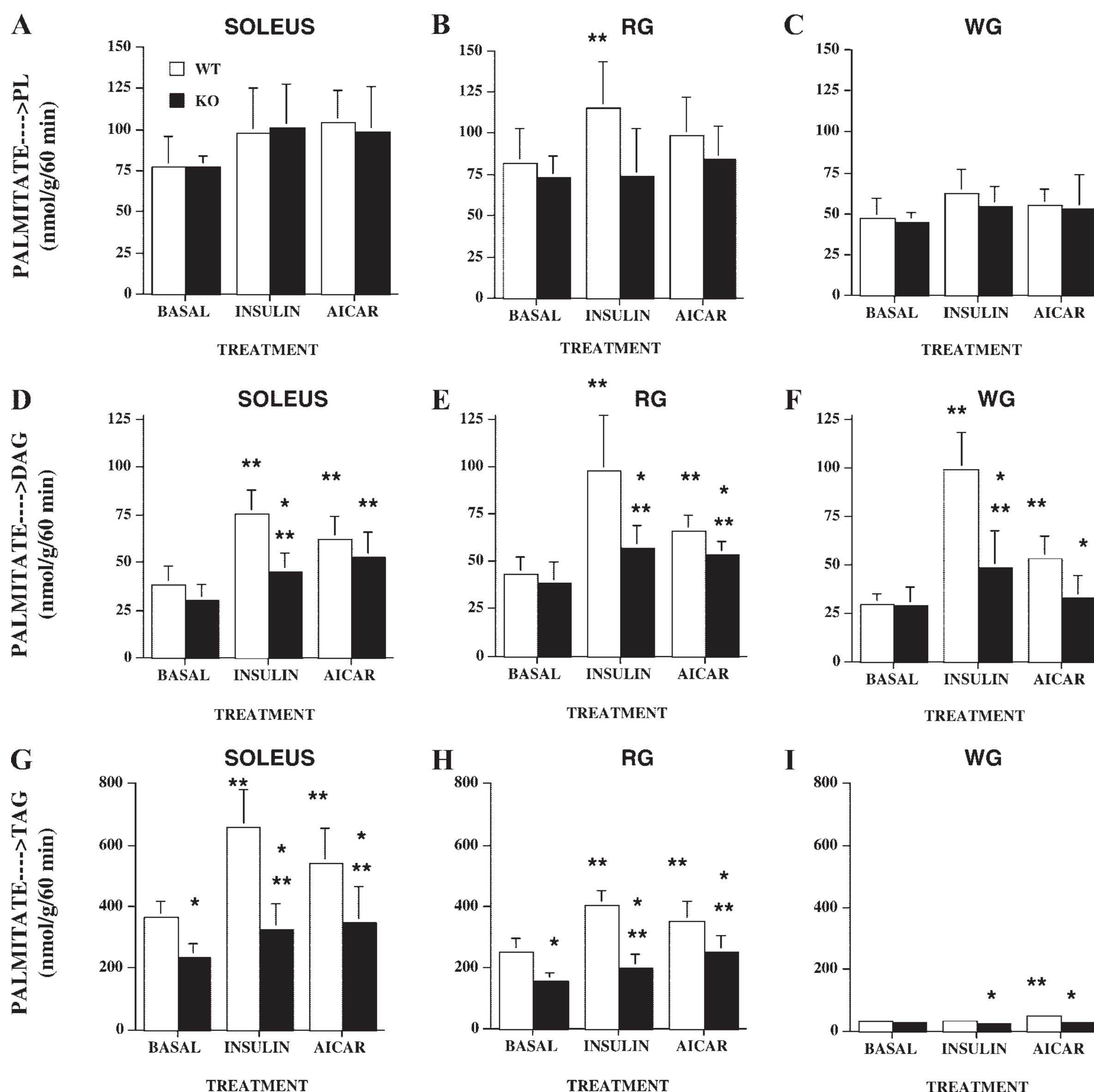

Fig. 5. Palmitate incorporation into intramuscular phospholipids (*A–C*), diacylglycerols (DAG; *D–F*), and triacylglycerols (TAG; *G–I*) under basal conditions, insulin stimulation, and AICAR stimulation in SOL, RG, and WG muscles of WT and KO mice. Data are means ± SE; n = 5–7 for each muscle in each group. *P < 0.05, KO vs. WT. **P < 0.05, insulin- or AICAR-stimulated vs. basal.

of KO mice (P < 0.05, Fig. 4*C*). With AICAR stimulation, the ACC phosphorylation attained was similar in the respective muscles in WT and KO mice (P < 0.05; Fig. 4*C*).

Palmitate Uptake and Incorporation into Intramuscular Phospholipids and Di- and Triacylglycerols

Palmitate uptake. Under basal perfusion conditions, palmitate uptake was reduced in KO mice (−23%, P < 0.05; Fig. 6). In WT mice, insulin (+60%) and AICAR (+77%) both stimulated palmitate uptake (P < 0.05; Fig. 6). In contrast, in KO mice there was only a small increase in insulin (21%)- and AICAR-stimulated (+13%) palmitate uptake (Fig. 6).

Phospholipids. No differences in palmitate incorporation into phospholipids were found between WT and KO mice (P > 0.05, Fig. 5, *A–C*).

Diacylglycerols. Under basal conditions, rates of palmitate incorporation into diacylglycerols did not differ in respective WT and KO muscles (P > 0.05, Fig. 5). AICAR stimulated palmitate incorporation into diacylglycerols in all WT (58–79%, P < 0.05; Fig. 5) and KO muscles (42–77%, P < 0.05; Fig. 5), except white gastrocnemius muscle in KO mice. The insulin-stimulated increase was far greater in WT muscles (+92 to +234%) than in KO muscles (+52 to +69%, P < 0.05; Fig. 5).

Triacylglycerols. As in WT mice, a large proportion of palmitate was synthesized into triacylglycerols. However, in KO mice, basal rates of triacylglycerol formation were reduced in soleus (−36%) and red gastrocnemius (−41%, P < 0.05; Fig. 5). The AICAR-stimulated rates of triacylglycerol formation were greater in WT muscles than in the respective KO muscles (P < 0.05, Fig. 5).

Downloaded from http://ajpendo.physiology.org/ by 10.220.33.3 on May 28, 2017

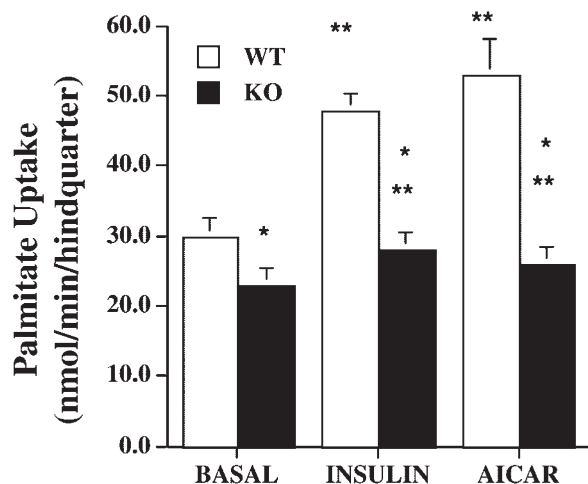

Fig. 6. Palmitate uptake by perfused hindlimb muscles in WT and KO mice. Data are means ± SE; $n = 5–7$ for each muscle in each group. *$P < 0.05$, KO vs. WT. **$P < 0.05$, insulin- or AICAR-stimulated vs. basal condition in the respective WT and KO groups.

Insulin stimulated the rate of palmitate incorporation into triacylglycerols in soleus (+80%) and red gastrocnemius (+59%) of WT mice ($P < 0.05$; Fig. 5). However, in KO mice, a much lower insulin stimulation occurred in soleus (+39%) and red gastrocnemius muscles (+27%).

Palmitate Oxidation

The basal rates of palmitate oxidation were reduced by 26% in KO mice compared with WT mice ($P < 0.05$, Fig. 7). Insulin reduced the rate of palmitate oxidation in both WT and KO mice ($P < 0.05$, Fig. 7). Although AICAR stimulated the rate of palmitate oxidation in WT mice (+100%), the AICAR-induced increase (+38%) in palmitate oxidation in KO mice was severely blunted ($P < 0.05$; Fig. 7).

Net Increase in Insulin-Stimulated Esterification and AICAR-Stimulated Oxidation

It is well known that the primary effect of insulin is the stimulation of palmitate incorporation into intramuscular triacylglycerols and that the primary effect of AICAR is the stimulation of fatty acid oxidation. To better appreciate the inhibition of these stimuli in FAT/CD36 null mice, we have compared the net increases in the insulin-stimulated triacylglycerol esterification (Fig. 8*A*) and in the AICAR-stimulated palmitate oxidation (Fig. 8*B*). The absolute increase in insulin-stimulated triacylglycerol esterfication (Δ = insulin − basal) was threefold lower in the soleus and red gastrocnemius of KO mice ($P < 0.05$; Fig. 8*A*). The absolute increase in AICAR-stimulated palmitate oxidation (Δ = AICAR − basal) was 3.7-fold lower in KO compared with WT mice ($P < 0.05$; Fig. 8*B*).

DISCUSSION

In the present study we have examined the effects of ablating the fatty acid transporter FAT/CD36 on palmitate metabolism in three metabolically heterogeneous types of skeletal muscle under basal conditions as well as during metabolic challenges with insulin and with AICAR. The present studies demonstrate that palmitate metabolism is markedly downregulated under basal conditions in FAT/CD36 null mice. This becomes even more apparent when muscles are challenged either with insulin or with AICAR. These metabolic effects are most pronounced in oxidative types of skeletal muscle, which have a greater capacity for fatty acid esterification and oxidation (18) and in which FAT/CD36 is most abundantly expressed (8, 35). Thus, in skeletal muscle, it appears that FAT/CD36 has a key regulatory role in fatty acid esterification and oxidation.

Fatty Acid Uptake by Skeletal Muscle

In recent years work in our laboratory has shown that fatty acid uptake into cardiac myocytes (32–35) and skeletal muscle (7, 8, 27, 33, 44) is a highly regulated, protein-mediated process. Early work suggested that FAT/CD36 was likely a key fatty acid transporter (8, 26, 35), which could be induced to translocate from an endosomal depot to the plasma membrane by insulin (12, 33, 34), muscle contraction (7, 32, 46), oligomycin (16, 32), and AICAR (12, 13, 32). In the present study we found that both insulin and AICAR stimulated fatty acid uptake in muscle of WT mice but that in muscles of FAT/CD36 null mice, fatty acid uptake was impaired under basal as well as insulin- and AICAR-stimulating conditions. It also appears that, relative to FAT/CD36, other fatty acid transporters (FABPpm, FATP1, and FATP4) contribute only minimally to increasing fatty acid uptake, despite the fact that FABPpm can be induced to translocate by AICAR (12). Insulin-induced translocation of FATP1 remains controversial (12, 38, 43, 49). Altogether, it appears that *1*) FAT/CD36 is important for maintaining basal rates of fatty acid uptake, and *2*) FAT/CD36 translocation to the plasma membrane is critically important for increasing the uptake of fatty acids when muscle is stimulated by insulin or by AICAR. This has important consequences for fatty acid esterification and oxidation.

Fatty Acid Metabolism in WT Muscle

The differences in palmitate esterification and oxidation in metabolically heterogeneous muscles of WT mice parallel

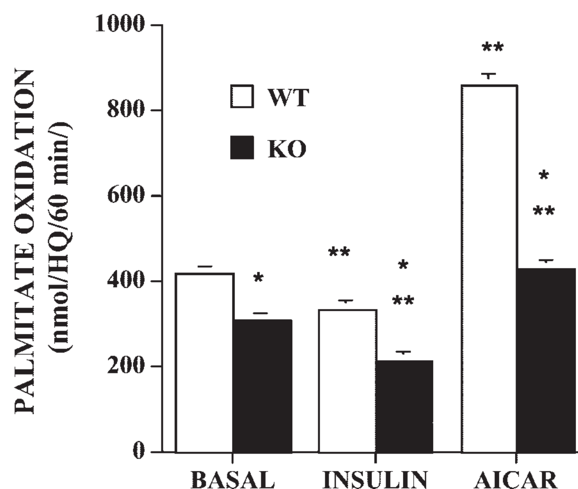

Fig. 7. Palmitate oxidation in WT and KO mice. Data are means ± SE; $n = 5–7$ for treatment in each group. Palmitate oxidation was examined in perfused mouse hindquarter (HQ) muscles under basal conditions, insulin stimulation, and AICAR stimulation. *$P < 0.05$, KO vs. WT. **$P < 0.05$, insulin- or AICAR-stimulated vs. basal.

Downloaded from http://ajpendo.physiology.org/ by 10.220.33.3 on May 28, 2017

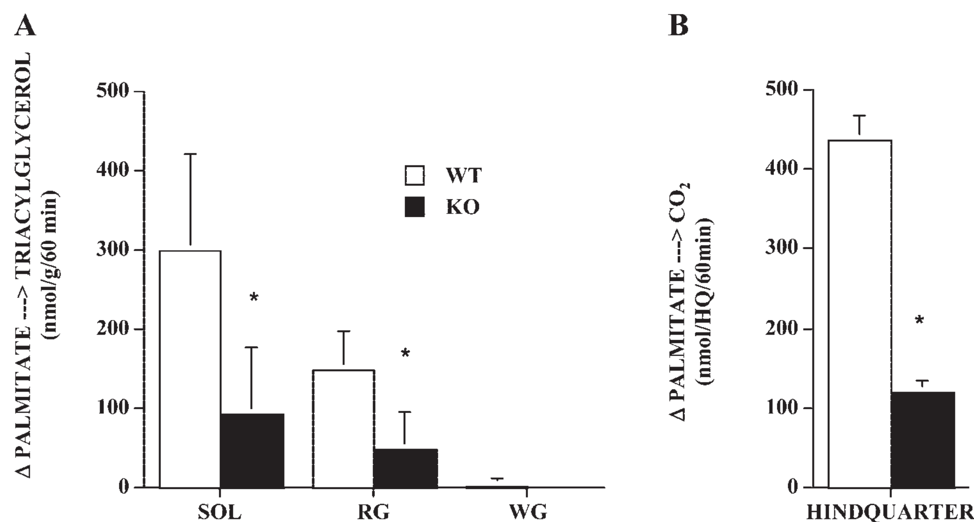

Fig. 8. Absolute net increases in insulin-stimulated rates of palmitate incorporation into intramuscular triacylglycerols (*A*) and AICAR-stimulated rates of palmitate oxidation (*B*) in SOL, RG, and WG muscles of WT and KO mice. Data are means ± SE; n = 5–7 for each muscle in each group in *A*; n = 5–7 for treatment in each group in *B*. Absolute net increase = insulin- or AICAR-stimulated rate − mean basal rate. *P < 0.05, KO vs. WT.

observations among similar muscles in rats (18). The responses to insulin and AICAR in muscles of WT mice concur with previous observation by our group in rat skeletal muscle in which insulin also stimulated fatty acid esterification (19, 33) and both contraction (17, 50) and AICAR (41, 42) stimulated fatty acid oxidation via AMPK activation.

Since the perfusion medium contains glucose, the reduction in skeletal muscle fatty acid oxidation during perfusion with insulin, observed in this and other studies (19), is attributable to the insulin-mediated increase in glucose transport into muscle (47, 50). The increased intracellular availability of glucose results in higher malonyl-CoA levels, which inhibit CPT1 and reduce the rates of fatty acid oxidation (47, 50).

The AICAR-induced increase in palmitate incorporation in triacylglycerols and glucose into glycogen is not without precedence in other situations in which rates of substrate oxidation are increased. For example, during muscle contraction, our group also found a net increase in palmitate incorporation into intramuscular triacylglycerols (17). Similarly, during treadmill exercise, our group observed an increase in glucose incorporation into glycogen (25). Under these conditions the uptake of substrates (palmitate and glucose) is known to be increased, and most of the palmitate and glucose taken up is oxidized. However, some is also seen to be synthesized into triacylglycerol (present study and Ref. 17) and glycogen (present study and Ref. 25). Despite this, these depots are concomitantly reduced during muscle contraction [i.e., degradation > synthesis (17, 25)], indicating that their turnover has increased . Thus, in the perfused hindlimb muscles, the AICAR-mediated upregulation of the rates of esterification and glycogenesis likely reflects an excess uptake of fatty acids and glucose, most of which are oxidized but some of which are esterified.

Metabolic Machinery for Stimulating Fatty Acid Metabolism is Not Impaired in FAT/CD36 Null Mice

The capacity for fatty acid oxidation in skeletal muscle was not impaired in FAT/CD36 null mice. Except for small reductions in citrate synthase activity, the metabolic machinery involved in fatty acid oxidation (ACC phosphorylation, CPT1, β-HAD, COX-4) was comparable in WT and KO mice. Diacylglycerol acyltransferase (DGAT), long-chain acyl-CoA synthetase (15), and GLUT4 (present study; data not shown) also do not differ in WT and KO mice. Insulin sensitivity with respect to glycogenesis (present study) and insulin-stimulated glucose uptake are not impaired in muscles of FAT/CD36 null mice (22). Altogether, the intracellular signaling and enzymatic machinery that is required to respond to either insulin or contraction-like stimuli do not differ appreciably in muscles of WT and KO mice. This makes the FAT/CD36 null model very suitable for determining the role of FAT/CD36 in fatty acid metabolism under basal as well as insulin- and AICAR-stimulating conditions.

Ablation of FAT/CD36 Accounts for Impaired Basal Rates of Fatty Acid Metabolism

The present study has shown that under basal conditions, fatty acid esterification and oxidation are diminished in FAT/CD36 null mice. This is largely attributable to the ablation of FAT/CD36, because a compensatory upregulation of other known fatty acid transporters (FABPpm, FATP1, FATP4) was not observed in either the red or white gastrocnemius muscles of FAT/CD36 null mice. However, there was an upregulation of FATP1 and FATP4 in the soleus muscle of FAT/CD36 KO mice, but these FATP1 and FATP4 increases appeared to have little effect, since they failed to normalize fatty acid esterification in the absence of FAT/CD36 in soleus muscle. In addition, the reduction in the basal rates of fatty acid oxidation by the hindlimb muscles of FAT/CD36 null mice is also associated with the ablation of FAT/CD36, despite the presence of other fatty acid transporters (FABPpm, FATP1, FATP4). Therefore, the evidence strongly indicates that reductions in the basal rates of palmitate esterification (∼ −40%) and oxidation (−26%) in skeletal muscle of FAT/CD36 null mice are the consequence of a reduced FAT/CD36-mediated fatty acid uptake.

FAT/CD36 is Critical for Upregulating Insulin- and AICAR-Stimulated Rates of Fatty Acid Metabolism

It is known that insulin stimulates palmitate uptake and esterification in skeletal muscle (19, 33). In the present study, the insulin-stimulated increases in the rate of fatty acid esterification into red muscle of FAT/CD36 mice were threefold

Downloaded from http://ajpendo.physiology.org/ by 10.220.33.3 on May 28, 2017

lower in FAT/CD36 null mice. In the highly glycolytic white gastrocnemius muscle, in which fatty acid metabolism is already very low, FAT/CD36 ablation had minimal impact. Since FAT/CD36 appears to be the primary fatty acid transporter that can be induced to translocate by insulin (12, 33, 34), and since in muscles of FAT/CD36 null mice the postreceptor insulin signaling machinery and the capacity for esterification in FAT/CD36 muscles are intact [i.e., normal insulin-stimulated rates of glycogenesis (present study) and insulin sensitivity (22), and normal DGAT and long-chain acyl-CoA synthetase activities (15)], it is highly likely that the impaired insulin stimulation of triacylglycerol formation in muscles of FAT/CD36 null mice is attributable to the marked reduction in palmitate transport into the muscle. This alone would limit substrate availability for palmitate esterification. Therefore, the present results strongly indicate that under normal conditions, the insulin-induced translocation of FAT/CD36 is responsible for the bulk of the fatty acids that are esterified.

FAT/CD36 ablation has a minimal effect on fatty acid incorporation into phospholipids, since under basal and stimulation conditions there were no differences between WT and KO mice. It may well be that preservation of membrane integrity is central to maintaining cellular viability, and therefore, the magnitude of the reduction in intracellular fatty acids associated with the ablation of FAT/CD36 may not be sufficient to reduce phospholipid synthesis.

Upregulation of fatty acid oxidation also has a large FAT/CD36-component, since the net change in AICAR-stimulated fatty acid oxidation was severely blunted in FAT/CD36 null mice. The modest upregulation of fatty acid oxidation may be attributable to the AMPK-stimulated translocation of FABPpm, since both AICAR (12) and muscle contraction (Han X-X and Bonen A, unpublished observations) induce the translocation of this transporter to the plasma membrane. As noted above, the signaling machinery and enzymatic capacity for fatty acid oxidation appeared to be essentially normal in FAT/CD36 null muscles. Previously, it has been shown that muscle contraction (7) and activation of AMPK, by AICAR (12) or oligomycin (32), upregulated fatty acid oxidation, and this also induces the translocation of FAT/CD36 from an intracellular depot to the plasma membrane, thereby increasing the rate of fatty acid transport. (12, 32). Therefore, the severe limitation in the ability to upregulate fatty acid oxidation in FAT/CD36 null mice would seem to be attributable to the reduced transport rate of fatty acids due to the ablation of FAT/CD36. This would limit fatty acid availability for oxidation.

There is, however, a caveat to our present findings. In recent studies it has been observed that FAT/CD36 is also present in mitochondria (2, 10, 24, 40), where it is associated with CPT1 (40) and appears to contribute to regulation of fatty acid oxidation in skeletal muscle (2, 10, 24, 40). Hence, we cannot ignore the possibility that the absence of FAT/CD36 at the mitochondrion may also have contributed to a reduced fatty acid oxidation in FAT/CD36 null mice. It is therefore important to determine whether the impaired fatty acid oxidation in muscle of FAT/CD36 null mice is largely attributable to a reduction in the rate of FAT/CD36-mediated fatty acid transport or to the absence of FAT/CD36 at the mitochondrion. Nevertheless, given the substantial impairment in the rate of insulin-stimulated esterification in FAT/CD36 null mice, which is most likely attributable to an impaired rate of palmitate transport, it would seem that a substantial component of the impaired fatty acid oxidation in FAT/CD36 null mice is also attributable to the impaired rate of fatty acid transport. Therefore, until the role of mitochondrial contribution of FAT/CD36 has been determined, it seems reasonable to propose that under normal conditions, the AICAR-stimulated translocation of FAT/CD36 is responsible for the bulk of the fatty acids that are oxidized.

In summary, the present study has shown that ablation of FAT/CD36 impairs basal as well as insulin- and AICAR-stimulated rates of palmitate esterification and oxidation in mouse skeletal muscles. FAT/CD36 ablation likely limits the quantity of fatty acids available for esterification and oxidation, particularly during metabolic challenges such as stimulation with insulin and with AICAR.

GRANTS

These studies were supported in part by grants from the Canadian Institutes of Health Research (to A. Bonen), the Natural Sciences and Engineering Research Council of Canada (to A. Bonen), the Heart and Stroke Foundation of Ontario (to A. Bonen), The Netherlands Heart Foundation Grant 2002.T049 (to J. Glatz), The Netherlands Organization for Health Research and Development Grant NWO-ZonMw 40-00812-98-03075 (to J. Luiken and J. Glatz), European Commission Integrated Project Grant LSHM-CT-2004-005272, Exgenesis (to J. Luiken and J. Glatz), National Heart, Lung, and Blood Institute Grants HL-70083 and HL-072942 (to M. Febbraio), and the Canada Research Chair Program (to A. Bonen).

J. Luiken is the recipient of a VIDI-Innovational Research Grant from the Netherlands Organization of Scientific Research (NWO-ZonMw Grant 016.036.305). J. F. C. Glatz is The Netherlands Heart Foundation Professor of Cardiac Metabolism. A. Bonen is the Canada Research Chair in Metabolism and Health.

REFERENCES

1. **Benton CR, Koonen DP, Calles-Escandon J, Tandon NN, Glatz JFC, Luiken JJFP, Heikkila JJ, Bonen A.** Differential effects of muscle contraction and PPAR agonists on the expression of fatty acid transporters in rat skeletal muscle. *J Physiol* 573: 199–210, 2006.
2. **Bezaire V, Bruce CR, Heigenhauser GJ, Tandon NN, Glatz JF, Luiken JJ, Bonen A, Spriet LL.** Identification of fatty acid translocase on human skeletal muscle mitochondrial membranes: essential role in fatty acid oxidation. *Am J Physiol Endocrinol Metab* 290: E509–E515, 2006.
3. **Binas B, Han XX, Eroll E, Luiken JJFP, Glatz JF, Dyck DJ, Motazavi R, Adihetty PJ, Hood DA, Bonen A.** A null mutation in H-FABP only partially inhibits skeletal muscle fatty acid metabolism. *Am J Physiol Endocrinol Metab* 285: E481–E489, 2003.
4. **Bonen A, Chabowski A, Luiken JJFP, Glatz JFC.** Mechanisms and regulation of protein-mediated cellular fatty acid uptake: molecular, biochemical and physiologic evidence. *Physiology*. 22:15–29, 2007.
5. **Bonen A, Dyck DJ, Ibrahimi A, Abumrad NA.** Muscle contractile activity increases fatty acid metabolism and transport and FAT/CD36. *Am J Physiol Endocrinol Metab* 276: E642–E649, 1999.
6. **Bonen A, Homonko D.** Effects of exercise and glycogen depletion on glyconeogenesis in muscle. *J Appl Physiol* 76: 1753–1758, 1994.
7. **Bonen A, Luiken JJFP, Arumugam Y, Glatz JFC, Tandon NN.** Acute regulation of fatty acid uptake involves the cellular redistribution of fatty acid translocase. *J Biol Chem* 275: 14501–14508, 2000.
8. **Bonen A, Luiken JJFP, Lui S, Dyck DJ, Kiens B, Kristiansen S, Turcotte L, van der Vusse GJ, Glatz JFC.** Palmitate transport and fatty acid transporters in red and white muscles. *Am J Physiol Endocrinol Metab* 275: E471–E478, 1998.
9. **Bonen A, Parolin ML, Steinberg GR, Calles-Escandon J, Tandon NN, Glatz JFC, Luiken JJFP, Heigenhauser GJF, Dyck DJ.** Triacylglycerol accumulation in human obesity and type 2 diabetes is associated with increased rates of skeletal muscle fatty acid transport and increased sarcolemmal FAT/CD36. *FASEB J* 18: 1144–1146, 2004.
10. **Campbell SE, Tandon NN, Woldegiorgis G, Luiken JJFP, Glatz JFC, Bonen A.** A novel function for fatty acid translocase (FAT)/CD36: involvement in long chain fatty acid transfer into the mitochondria. *J Biol Chem* 279: 36325–36341, 2004.

Downloaded from http://ajpendo.physiology.org/ by 10.220.33.3 on May 28, 2017

11. **Chabowski A, Coort SL, Calles-Escandon J, Tandon NN, Glatz JF, Luiken JJ, Bonen A.** Insulin stimulates fatty acid transport by regulating expression of FAT/CD36 but not FABPpm. *Am J Physiol Endocrinol Metab* 287: E781–E789, 2004.
12. **Chabowski A, Coort SLM, Calles-Escandon J, Tandon NN, Glatz JFC, Luiken JJFP, Bonen A.** The subcellular compartmentation of fatty acid transporters is regulated differently by insulin and by AICAR. *FEBS Lett* 579: 2428–2432, 2005.
13. **Chabowski A, Momken I, Coort SLM, Calles-Escandon J, Tandon NN, Glatz JFC, Luiken JJFP, Bonen A.** Prolonged AMPK activation increases the expression of fatty acid transporters in cardiac myocytes and perfused hearts. *Mol Cell Biochem* 288: 201–212, 2006.
14. **Clarke DC, Miskovic D, Han XX, Calles-Escandon J, Glatz JFC, Luiken JJFP, Heikkila JJ, Bonen A.** Overexpression of membrane-associated fatty acid binding protein (FABPpm) in vivo increases fatty acid sarcolemmal transport and metabolism. *Physiol Genomics* 17: 31–37, 2004.
15. **Coburn CT, Knapp FF Jr, Febbraio M, Beets AL, Silverstein RL, Abumrad NA.** Defective uptake and utilization of long chain fatty acids in muscle and adipose tissue of CD36 knockout mice. *J Biol Chem* 275: 32523–32529, 2000.
16. **Coort SLM, Hasselbaink DM, Koonen DYP, Willems J, Coumans WA, Chabowski A, van der Vusse GJ, Bonen A, Glatz JFC, Luiken JJFP.** Enhanced sarcolemmal FAT/CD36 content and triacylglycerol storage in cardiac myocytes from obese Zucker rats. *Diabetes* 53: 1655–1663, 2004.
17. **Dyck DJ, Bonen A.** Muscle contraction increases palmitate esterification and oxidation and triacylglycerol oxidation. *Am J Physiol Endocrinol Metab* 275: E888–E896, 1998.
18. **Dyck DJ, Peters SJ, Glatz J, Gorski J, Keizer H, Kiens B, Liu S, Richter EA, Spriet LL, van der Vusse GJ, Bonen A.** Functional differences in lipid metabolism in resting skeletal muscle of various fiber types. *Am J Physiol Endocrinol Metab* 272: E340–E351, 1997.
19. **Dyck DJ, Steinberg G, Bonen A.** Insulin increases FFA uptake and esterification but reduces lipid utilization in isolated contracting muscles. *Am J Physiol Endocrinol Metab* 281: E600–E607, 2001.
20. **Febbraio M, Abumrad NA, Hajjar DP, Sharma K, Cheng W, Frieda S, Pearce A, Silverstein RL.** A null mutation in murine CD36 reveals an important role in fatty acid and lipoprotein metabolism. *J Biol Chem* 274: 19055–19062, 1999.
21. **Fueger PJ, Hess HS, Posey KA, Bracy DP, Penceck RR, Charron MJ, Wasserman DH.** Control of exercise-stimulated muscle glucose uptake by GLUT4 is dependent on glucose phosphorylation capacity in the conscious mouse. *J Biol Chem* 279: 50956–50961, 2004.
22. **Goudriaan JR, Dahlmans VE, Teusink B, Ouwens DM, Febbraio M, Maassen JA, Romijn JA, Havekes LM, Voshol PJ.** CD36 deficiency increases insulin sensitivity in muscle, but induces insulin resistance in the liver in mice. *J Lipid Res* 44: 2270–2277, 2003.
23. **Han XX, Bonen A.** Epinephrine translocates GLUT-4 but inhibits insulin-stimulated glucose transport in rat muscle. *Am J Physiol Endocrinol Metab* 274: E700–E707, 1998.
24. **Holloway GP, Bezaire V, Heigenhauser GJ, Tandon NN, Glatz JF, Luiken JJ, Bonen A, Spriet LL.** Mitochondrial long chain fatty acid oxidation, fatty acid translocase/CD36 content and carnitine palmitoyltransferase 1 activity in human skeletal muscle during aerobic exercise. *J Physiol* 571: 201–210, 2006.
25. **Hutber CA, Bonen A.** Glycogenesis in muscle and liver during exercise. *J Appl Physiol* 66: 2811–2817, 1989.
26. **Ibrahimi A, Bonen A, Blinn WD, Hajri T, Li X, Zhong K, Cameron R, Abumrad NA.** Muscle-specific overexpression of FAT/CD36 enhances fatty acid oxidation by contracting muscles, reduces plasma triglycerides and fatty acids, and increases plasma glucose and insulin. *J Biol Chem* 274: 26761–26766, 1999.
27. **Koonen DPY, Benton CR, Arumugam Y, Tandon NN, Calles-Escandon J, Glatz JFC, Luiken JJFP, Bonen A.** Different mechanism can alter fatty acid transport when muscle contractile activity is chronically altered. *Am J Physiol Endocrinol Metab* 286: E1042–E1049, 2004.
28. **Koves TR, Noland RC, Bates AL, Henes ST, Muoio DM, Cortright RN.** Subsarcolemmal and intermyofibrillar mitochondria play distinct roles in regulating skeletal muscle fatty acid metabolism. *Am J Physiol Cell Physiol* 288: C1074–C1082, 2005.
29. **Lemieux K, Han XX, Dombrowski L, Bonen A, Marette A.** The transferrin receptor defines two distinct contraction-responsive GLUT4 vesicle populations. *Diabetes* 49: 183–189, 2000.
30. **Lowry OH, Passonneau JV.** *A Flexible System of Enzymatic Analysis*. New York: Academic, 1972.
31. **Luiken JJFP, Arumugam Y, Dyck DJ, Bell RC, Pelsers ML, Turcotte LP, Tandon NN, Glatz JFC, Bonen A.** Increased rates of fatty acid uptake and plasmalemmal fatty acid transporters in obese Zucker rats. *J Biol Chem* 276: 40567–40573, 2001.
32. **Luiken JJFP, Coort SML, Willems J, Coumans WA, Bonen A, van der Vusse GJ, Glatz JFC.** Contraction-induced fatty acid translocase/CD36 translocation in rat cardiac myocytes is mediated through AMP-activated protein kinase signaling. *Diabetes* 52: 1627–1634, 2003.
33. **Luiken JJFP, Dyck DJ, Han XX, Tandon NN, Arumugam Y, Glatz JFC, Bonen A.** Insulin induces the translocation of the fatty acid transporter FAT/CD36 to the plasma membrane. *Am J Physiol Endocrinol Metab* 282: E491–E495, 2002.
34. **Luiken JJFP, Koonen DPY, Willems J, Zorzano A, Fischer Y, van der Vusse GJ, Bonen A, Glatz JFC.** Insulin stimulates long-chain fatty acid utilization by rat cardiac myocytes through cellular redistribution of FAT/CD36. *Diabetes* 51: 3113–3119, 2002.
35. **Luiken JJFP, Turcotte LP, Bonen A.** Protein-mediated palmitate uptake and expression of fatty acid transport proteins in heart giant vesicles. *J Lipid Res* 40: 1007–1016, 1999.
36. **Nickerson J, Bonen A.** Defining a role for skeletal muscle fatty acid transport proteins. *2nd Northern Lights Conference. Canadian Federation of Biological Societies*, Guelph, Ontario, Canada, 2005, p. F47.
37. **Peters SJ, Dyck DJ, Bonen A, Spriet LL.** Effect of epinephrine on lipid metabolism in resting skeletal muscle. *Am J Physiol Endocrinol Metab* 275: E471–E4778, 1998.
38. **Richards MR, Harp JD, Ory DS, Schaffer JE.** Fatty acid transport protein 1 and long-chain acyl coenzyme A synthetase 1 interact in adipocytes. *J Lipid Res* 47: 665–672, 2006.
39. **Ryder JW, Kawano Y, Chibalin AV, Rincon J, Tsao T, Stenbit A, Combatsiaris T, Yang J, Holamn GD, Charron M, Zierath JR.** In vitro analysis of glucose-transport system in GLUT4-null skeletal muscle. *Biochem J* 342: 321–328, 1999.
40. **Schenk S, Horowitz JF.** Coimmunoprecipitation of FAT/CD36 and CPTI in skeletal muscle increases proportionally with fat oxidation after endurance exercise training. *Am J Physiol Endocrinol Metab* 291: E254–E260, 2006.
41. **Smith AC, Bruce CR, Dyck DJ.** AMP kinase activation with AICAR further increases fatty acid oxidation and blunts triacylglycerol hydrolysis in contracting rat soleus muscle. *J Physiol* 565: 547–553, 2005.
42. **Smith AC, Bruce CR, Dyck DJ.** AMP kinase activation with AICAR simultaneously increases fatty acid and glucose oxidation in resting rat soleus muscle. *J Physiol* 565: 537–546, 2005.
43. **Stahl A, Evans JG, Pattel S, Hirsch D, Lodish HF.** Insulin causes fatty acid transport protein translocation and enhanced fatty acid uptake in adipocytes. *Dev Cell* 2: 477–488, 2002.
44. **Steinberg GR, Dyck DJ, Calles-Escandon J, Tandon NN, Luiken JJFP, Glatz JF, Bonen A.** Chronic leptin administration decreases fatty acid uptake and fatty acid transporters in rat skeletal muscle. *J Biol Chem* 277: 8854–8860, 2002.
45. **Stenbit AE, Tsao TS, Li J, Burcelin R, Greenen DL, Factor SM, Houseknecht K, Katz EB, Charron MJ.** GLUT4 heterozygous knockout mice develop insulin resistance and diabetes. *Nat Med* 3: 1096–1101, 1997.
46. **Turcotte LP, Raney MA, Todd MK.** ERK1/2 inhibition prevents contraction-induced increase in plasma membrane FAT/CD36 content and FA uptake in rodent muscle. *Acta Physiol Scand* 184: 131–139, 2005.
47. **Turcotte LP, Swenberger JR, Yee AJ.** High carbohydrate availability increases LCFA uptake and decreases LCFA oxidation in perfused muscle. *Am J Physiol Endocrinol Metab* 282: E177–E183, 2002.
48. **Veerkamp JH, van Moerkerk TB, Glatz JF, Zuurveld JG, Jacobs AE, Wagenmakers AJ.** $^{14}CO_2$ production is no adequate measure of [^{14}C]fatty acid oxidation. *Biochem Med Metab Biol* 35: 248–259, 1986.
49. **Wu Q, Ortegon AM, Tsang B, Doege H, Feingold KR, Stahl A.** FATP1 is an insulin-sensitive fatty acid transporter involved in diet-induced obesity. *Mol Cell Biol* 26: 3455–3467, 2006.
50. **Yee AJ, Turcotte LP.** Insulin fails to alter plasma LCFA metabolism in muscle perfused at similar glucose uptake. *Am J Physiol Endocrinol Metab* 283: E73–E77, 2002.

Downloaded from http://ajpendo.physiology.org/ by 10.220.33.3 on May 28, 2017