Muscle-specific Overexpression of FAT/CD36 Enhances Fatty Acid Oxidation by Contracting Muscle, Reduces Plasma Triglycerides and Fatty Acids, and Increases Plasma Glucose and Insulin*

(Received for publication, March 11, 1999, and in revised form, July 5, 1999)

Azeddine Ibrahimitä, Arend Bonenb, W. Dennis Blinnb, Tahar Hajrić, Xin Li†, Kai Zhong†, Roger Cameron†, and Nada A. Abumrad‡‡***

From the ‡Department of Physiology and Biophysics, State University of New York, Stony Brook, New York 11794-8661, the §Department of Kinesiology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada, the ¶Department of Chemistry, State University of New York, Stony Brook, New York 11794-3400, and the ¶¶Chemistry Department, Brookhaven National Laboratory, Upton, New York 11973

Increasing evidence has implicated the membrane protein CD36 (FAT) in binding and transport of long chain fatty acids (FA). To determine the physiological role of CD36, we examined effects of its overexpression in muscle, a tissue that depends on FA for its energy needs and is responsible for clearing a major fraction of circulating FA. Mice with CD36 overexpression in muscle were generated using the promoter of the muscle creatine kinase gene (MCK). Transgenic (MCK-CD36) mice had a slightly lower body weight than control litter mates. This reflected a leaner body mass with less overall adipose tissue, as evidenced by magnetic resonance spectroscopy. Soleus muscles from transgenic animals exhibited a greatly enhanced ability to oxidize fatty acids in response to stimulation/contraction. This increased oxidative ability was not associated with significant alterations in histological appearance of muscle fibers. Transgenic mice had lower blood levels of triglycerides and fatty acids and a reduced triglyceride content of very low density lipoproteins. Blood cholesterol levels were slightly lower, but no significant decrease in the cholesterol content of major lipoprotein fractions was measured. Blood glucose was significantly increased, while insulin levels were similar in the fed state and higher in the fasted state. However, glucose tolerance curves, determined at 20 weeks of age, were similar in control and transgenic mice. In summary, the study documented, in vivo, the role of CD36 to facilitate cellular FA uptake. It also illustrated importance of the uptake process in muscle to overall FA metabolism and glucose utilization.

Our previous work with rat adipocytes presented evidence that membrane transport of long chain fatty acids (FA)† had a protein-facilitated component (1, 2). We identified an 88-kDa glycoprotein as a candidate FA transporter by labeling with inhibitors of FA transport, notably sulfo-N-succinimidyl derivatives of long chain FA (2, 3). The cDNA, isolated from a rat adipose tissue cDNA library (4), coded for a protein (FAT, for FA translocase), which is the rat homolog of human platelet CD36 (5) and of bovine mammary PASIV (6). FAT/CD36 mRNA is abundant in tissues active in FA metabolism such as heart, skeletal muscle, fat, and intestines (4, 7), and modulated by conditions that alter lipid metabolism, such as diabetes mellitus and high fat feeding (8). In preadipocytes, the mRNA is induced by FA (9, 10), an effect mediated by the nuclear transcription factors peroxisome proliferator-activated receptors (10, 11), which play a role in adipocyte differentiation and possibly obesity (11, 12). CD36 mRNA is also a marker of preadipocyte differentiation, and its early induction, is paralleled with an increase in membrane FA transport (4). More recently, CD36 has been identified as a causal gene at the peak of linkage for defects in FA and glucose metabolism in spontaneously hypertensive rats (13), a rodent model of insulin resistance.

There is indirect evidence to support the role of CD36 in muscle FA uptake. In rats, expression is higher in oxidative as opposed to glycolytic muscles (14), and there is good correlation between CD36 levels and relative rates of FA transport (15). In humans, several studies, using an iodinated FA derivative, BMIPP (16), reported an association between defective myocardial FA uptake, hypertrophic cardiac myopathy, and deficiency of CD36 (17, 18).

To directly assess the role of CD36 in mediating muscle FA uptake, in vivo, we generated a transgenic mouse model with overexpression of CD36 targeted to muscle tissues. Muscle is a primary FA consumer and derives a large fraction of the energy needed from FA oxidation. In addition, the role that muscle mass plays in FA clearance and in FA-mediated development of insulin resistance is well established (19). Based on this, we hypothesized that if CD36 determined FA uptake and utilization by muscle, its overexpression in this tissue should play a uniquely important role in regulating metabolism in general. We report that muscle-targeted overexpression of CD36 was associated with enhanced muscle FA oxidation and with alterations in body fat and in blood levels of FA, triglycerides, cholesterol, glucose, and insulin.

**EXPERIMENTAL PROCEDURES**

Materials—The [α-32P]dCTP was purchased from ICN. The nylon (Hybond N+) membranes and the enhanced chemiluminescence immunodetection system (ECL) were from Amersham Pharmacia Biotech. Enzymes for RNA and DNA manipulation and the random-primed kit were from Roche Molecular Biochemicals. RNA STAT-60 kit was from Tel-Test Inc. (Friendswood, TX). The polyclonal CD36 antibody was generated in rabbits against rat adipocyte CD36, and the monoclonal...
antibody against human CD36 was a kind gift from Dr. Narendra Tandon. Kodak films, FA, and all other chemical products were from Sigma.

**Generation of CD36 Transgenic Mice and DNA Analysis—** Cloning was performed according to Sambrook et al. (20). A 1.7-kb DNA fragment containing a 1.3-kb PstI sequence and 3′ flanking sequence was cloned in pBluescript under the control of the regulatory sequences (3.3 kb) of the mouse creatine kinase gene (MCK). The procedure was as described by Levak-Frank et al. (21) for muscle over expression of lipoprotein lipase. The linear MCK-CD36 minigene, prepared by cutting the plasmid with HindIII and SsiI, was injected into the male pronucleus of fertilized eggs from superovulated females of the FVB line that had been mated with males from the same genetic background. Microinjected eggs were transferred into the oviducts of surrogate females. Transgenic mice were identified by Southern blot screening of genomic DNA from tail biopsies. The DNA was digested with PstI and probed with radiolabeled 1.3-kb CD36 cDNA. Mice homozygous for the transgene (identified by litters that screened 100% positive) were generated. Preliminary tests showed similar blood profiles of homozygotes and heterozygotes, and only heterozygotes were used for the experiments reported in this study. Controls (negative for the transgene) were derived from the litter of heterozygote parents. In some cases the mice (transgenic and controls) used for experiments were from the same litter. In other cases, they were from different litters, but were matched for age, sex, and, whenever possible, litter size. Mice were used for studies between 16 and 20 weeks of age.

**RNA Analysis—** Tissues were excised from euthanized mice, rinsed with phosphate-buffered saline, and frozen in liquid nitrogen. Tissue samples (100 mg) were Dounce-homogenized in STAT-60 buffer, as described by Chomczynski and Sacchi (22) and using the RNA STAT-60 kit. The RNA, electrophoresed on denaturing agarose gel, was trans- ferred to Hybond-N membrane, and probed with a 32P-labeled DNA probes (10^6 cpm/ml) (4). mRNA for glyceraldehyde-3-phosphate dehydrogenase was used as internal standard.

**Protein Analysis—** Muscle or heart tissue was homogenized in 1 ml of ice-cold TES buffer (20 mm Tris, 1 mm EDTA, 250 mm sucrose). The homogenate was centrifuged (16,000 × g) to yield a pellet P1 and a supernatant, S1. P1 was layered on 38% sucrose and centrifuged at 105,000 × g for 30 min) by electrical stimulation (150 ms trains of 0.1-ms impulses (24, 25) and thin layer chromatography (silica GF 250 microns, Analtech, Newark, DE) of the extract (50 μl).

**Plasma Parameters—** Control and transgenic mice were fed a chow diet ad libitum. Blood was taken from fed or 12-h fasted animals into EDTA-rinsed capillaries, and plasma was immediately prepared. Cholesterol, triglycerides, and glucose were determined using enzymatic test kits from Sigma. FFA were measured using the Wako kit (Wako Chemicals, Richmond, VA) and insulin using a kit from Linco Research Inc. (St. Louis, MO).

For lipoprotein determination, plasma was pooled after centrifugation of blood samples (1000 × g, 20 min, 4°C) and subjected to ultracentrifugation (36,000 rpm, SW 41 rotor, 48 h, 12°C) in a saline potas- sium bromide gradient (26). Fractions were collected by density (grams/milliliter); very low density lipoproteins and intermediate density lipoproteins (VLDL + IDL), d < 1.018; low density lipoproteins (LDL), 1.018 < d < 1.063; high density lipoproteins (HDL), 1.063 < d < 1.210.

**Glucose Tolerance Tests—** Following a 4-h fast, mice were injected via a lateral tail vein with 100 μl of b-glucose solution (1 mg/g of body weight). Blood was obtained from a small blunt cut at the tip of the tail and glucose was measured using a Precision Q.I.D. monitoring system before (0) and 5, 15, 30, 60, and 120 min after glucose administration (27).

**RESULTS**

**Generation of a Transgenic Mouse Model That Overexpresses CD36 in Muscle Tissues—** Fig. 1A shows a Southern blot of DNA from animals positive for the CD36 minigene, while signal was undetectable in DNA from controls. Controls were animals negative for the transgene and were derived from heterozygote parents. B and C show that muscle tissues from transgenic animals exhibited enhanced expression of CD36 mRNA (3–4-fold) and protein (2–4-fold) as compared with control littermates. mRNA levels for lipoprotein lipase and glycer- aldehyde-3-phosphate dehydrogenase were examined, as internal controls, in muscle tissues from control and transgenic mice and found not to be altered. Tissues other than muscle (adipose, liver) from MCK-CD36 mice did not show alterations in CD36 expression (data not shown).

MCK-CD36 mice developed normally with no incidence of disease noted, but they exhibited smaller body weight than littermate controls during the first 6 months of growth. For male mice (Fig. 2A), the difference ranged from 13 to 15% and was significant throughout the 14 week growth curve. It was of smaller magnitude for females and significant only during early growth (Fig. 2B). Litter size, which can determine pup nutrition and early development, was taken into consideration in the weight comparison by matching litter size distribution in both groups.

Magnetic resonance images of control and transgenic mice derived from the same litter or from similar size litters showed that the MCK-CD36 mice had less overall body fat (Fig. 3A). The mice sets compared were matched for age (16–20 weeks) and sex. The difference in body fat content was apparent at all time points from the abdomen region to the tail of the animal. Distribution of fat tissue did not appear to be altered. Fig. 3B compares images of calves from control (panel a) and MCK-CD36 (panel b) 16-week-old male mice. The region of interest spectrum from the MCK-CD36 leg shows a lipid/water ratio of 2% as compared with 7% in the case of the control (Fig. 3C). These spectra are expanded in Fig. 3D.

**Light and Electron Microscopy of Muscle (Data Not Shown)—** Light microscopic examination of muscle tissues from control and transgenic mice showed similar fiber structure and organization and no evidence of neutral lipid depositions in cross-sections of myocardium or skeletal muscle, stained with Oil Red O.
Electron microscopy on two sets of samples from cardiac and skeletal muscles identified no changes in appearance of cell organelles or in number of mitochondria.

FA Metabolism by Muscle Tissues—Palmitate oxidation by soleus muscle, incubated in vitro, was compared for a group of control and MCK-CD36 mice (16–20 weeks old). Oxidation by the muscle at rest was similar for both groups. It increased in response to stimulation-induced contraction. The increase averaged 1.9-fold in muscles from control mice, which was in line with previous observations (24, 25). In contrast, the increase averaged 5.6-fold in MCK-CD36 muscles, indicating a greatly enhanced capacity to increase FA oxidation in response to contraction (Table I). The magnitude of the increase varied between MCK-CD36 mice, most likely reflecting differences in degree of CD36 overexpression. Despite this variability, in all cases, soleus muscles from transgenic mice exhibited a higher ability to oxidize FA in response to contraction than the corresponding muscles from matched controls. FA incorporation into cellular lipids (monoglycerides, diglycerides, triglycerides, and skeletal muscle from transgenic mice. Protein levels were detected using a monoclonal antibody against CD36 or (not shown) a polyclonal antibody against rat adipose CD36. Reaction with preimmune sera did not yield a detectable signal (not shown). Immunodetection was by the ECL kit (Amersham Pharmacia Biotech). Films were scanned and mean values are shown ± S.E.
Smaller decreases were noted in other lipoprotein fractions.

Plasma FA (Table II) were lower in transgenic mice with the difference most apparent after an overnight fast (p < 0.01) as FA increased with fasting in controls but not in transgenic mice.

Plasma cholesterol (Table II) was slightly lower (about 11%) in transgenic mice as compared with control litter mates (p < 0.05). Cholesterol levels were not significantly changed by fasting, so data were pooled. No significant change was measured in cholesterol content of lipoprotein fractions (VLDL, LDL, and HDL) from transgenic mice (data not shown).

In contrast to the decreases observed in all blood lipids, glucose levels were increased by about 30% in transgenic mice (Table II) in both the fed and fasted states. Insulin levels were not significantly different in fed mice but increased about 2-fold after an overnight fast (Table II). However, MCK-CD36 mice, tested at 20 weeks of age, did not exhibit clear signs of poor glucose tolerance. As shown in Fig. 5, following an intravenous glucose load (1 mg/g), plasma glucose levels peaked and then declined in a similar fashion in control and MCK-CD36 mice.

**DISCUSSION**

This study examined the metabolic effects of overexpressing CD36 in muscle tissues. Several conclusions can be drawn from the findings. First, CD36 expression determines FA utilization by a tissue, in this case muscle. Second, CD36 level in muscle can impact lean body mass and blood lipids, most notably FA and triglycerides. Third, CD36 expression, by determining muscle FA utilization, can modulate glucose and insulin levels.

The main conclusion of the studies described is that CD36, *in vivo*, functions as a transporter of long chain FA by muscle, and the transport step rate-limits and determines FA utilization. Muscle overexpressing CD36 had an increased capacity for uptake and oxidation of blood FA, which reduced the FA available for hepatic triglyceride synthesis. As a result, MCK-CD36 mice exhibited lower levels of total and VLDL triglycerides and a greater decrease in body fat. FA oxidation by muscle from control animals increased 1.9-fold in response to contraction while that from transgenics increased 5.6-fold. The increase in control muscles is consistent with the previous finding that long term (7 days), low frequency-stimulated muscle contraction produced 2-fold increases in both membrane CD36 expression and FA transport across sarcolemmal vesicles (14). The larger increase (5.6-fold) in FA oxidation by muscle overexpressing CD36 indicates that oxidation is rate-limited by FA uptake at the plasma membrane. So increasing transport capacity allows parallel increases in oxidative capacity. Although CD36 overexpression did not alter FA oxidation by the resting soleus, this may reflect the fact that resting muscle, *in vitro*, is under little tension, and its FA metabolism may be low enough so as not to be limited by uptake, a situation unlikely to be encountered physiologically. FA oxidation by the resting soleus in our studies was lower than estimates obtained from CO2 release by the mixed muscles of the perfused hindlimb (28) (0.5 nmol/g/min versus about 2 nmol/g/min). An alternative explanation is that most of the CD36 pool in muscle at rest is intracellular and is recruited by contraction into the plasma membrane, as was demonstrated for the glucose transporter, Glut4 (29). However, no other evidence suggests such a mechanism.

The data in soleus muscle highlight a tight coupling between FA transport and oxidation. CD36 overexpression increased palmitate oxidation by contracting muscle but not its esterification into neutral lipids, which is consistent with findings from the magnetic resonance imaging and histology indicating no increase and possibly a decrease in intramuscular lipid depots in muscle from transgenic mice (Fig. 3). FA esterifica-

---

**Fig. 3. Nuclear magnetic resonance images of control and transgenic mice.**

A, representative, transverse, T1-weighted spin echo images of control (panel a) and MCK-CD36 (panel b) mice showing fat tissue as bright in contrast to other tissues. Slices (1-mm thickness, separated by 1 mm) proceed from the abdomen to the tail (top left to bottom right). B, images of the right calves for a control (panel a) and MCK-CD36 mouse (panel b) with the region of interest spectra shown in C. Major spectra on the left correspond to water, the minor to lipid. D, expanded lipid portions of C. The spectra shown are from 16-week-old males derived from the same litter. Data shown are typical for scans performed on five mice (16–20 weeks old) from each group, matched for age and litter size.

**Blood Parameters**—Plasma triglycerides (TG) were about 30% (p < 0.001) lower in transgenic mice (Table II). Fasting lowered TG in control and transgenic mice to a similar extent so the difference between the groups persisted (p < 0.001). The decrease in blood TG in MCK-CD36 mice was pronounced in the VLDL fraction, which lost 40% of its TG content (Fig. 4).
tion may increase when oxidation is impaired or when it is over saturated by FA supply. Accumulation of FA-acyl-intermediates may then stimulate triglyceride synthesis, possibly at the level of the key enzyme, diglyceride acyltransferase.

The findings in transgenic MCK-CD36 mice add to an increasing body of evidence supporting the role of CD36 in FA transport. The protein, purified from adipose tissue, bound long chain FA in vitro (30). Expression of the coding sequence of FAT/CD36 in the sense and antisense orientation in mammalian cells (fibroblasts and preadipocytes, respectively) resulted in the expected changes in FA transport (31, 32). Important recent evidence was contributed with generation of mice null for CD36 by Febbraio et al. (33). The mice exhibited a lipid profile, which was opposite to the one observed in MCK-CD36 mice, with significant increases in blood FA, TG, and cholesterol. Isolated adipocytes from CD36 null mice lacked the high affinity saturable component of FA transport.

The increase in blood glucose levels in MCK-CD36 mice may have reflected the combined effects of FA-induced sparing of glucose utilization by muscle as well as those resulting from possible alterations in insulin secretion and hepatic glucose production. FA stimulate basal insulin secretion at the same time that they decrease beta cell insulin stores (34). In muscle tissues, FA inhibit insulin stimulation of glucose transport and phosphorylation (35). It would appear that resistance to insulin was not the underlying cause for the hyperglycemia and for the 2-fold higher insulin in fasted transgenic mice at the age we tested them (20 weeks). However, it remains to be determined if MCK/CD36 mice will develop glucose intolerance as they advance in age or as they are challenged by high fat diets. In

### Table I

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MCK-CD36</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxidation</strong></td>
<td>15.1 ± 5.2</td>
<td>22.2 ± 3.9</td>
</tr>
<tr>
<td><strong>TG</strong></td>
<td>87.3 ± 13.7</td>
<td>76.1 ± 12.4</td>
</tr>
<tr>
<td><strong>DG</strong></td>
<td>14.2 ± 1.9</td>
<td>13.0 ± 1.4</td>
</tr>
<tr>
<td><strong>MG</strong></td>
<td>1.3 ± 0.13</td>
<td>1.2 ± 0.17</td>
</tr>
<tr>
<td><strong>PL</strong></td>
<td>15.4 ± 1.5</td>
<td>14.4 ± 1.3</td>
</tr>
</tbody>
</table>

* p < 0.05, n = 6, four males and two females in each group.

### Table II

<table>
<thead>
<tr>
<th></th>
<th>Fasted</th>
<th>Fed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TG (mg/dl)</strong></td>
<td>137.6 ± 10.8</td>
<td>173.6 ± 13.1</td>
</tr>
<tr>
<td><strong>FFA (mEq/liter)</strong></td>
<td>1.04 ± 0.09</td>
<td>0.76 ± 0.06</td>
</tr>
<tr>
<td><strong>Ch (mg/dl)</strong></td>
<td>147.4 ± 4.1</td>
<td>130.5 ± 6.2</td>
</tr>
<tr>
<td><strong>Glc (mg/dl)</strong></td>
<td>118.7 ± 7.5</td>
<td>171.7 ± 6.8</td>
</tr>
<tr>
<td><strong>Ins (ng/dl)</strong></td>
<td>0.20 ± 0.028</td>
<td>0.36 ± 0.030</td>
</tr>
</tbody>
</table>

* p < 0.001, n = 12 for control and transgenic mice in both fed and fasted states.

### Figures

**Fig. 4.** Triglyceride content of major lipoproteins in control and MCK-CD36 mice. Mice (16–20 weeks old) were fasted for 12 h. Blood was collected from the tail vein into EDTA-coated capillary tubes and centrifuged to separate out plasma, which was used to assay triglyceride content of major lipoproteins following separation by density ultracentrifugation. Values shown are means ± S.E. *p < 0.001, n = 10.

**Fig. 5.** Glucose clearance in response to a glucose load. Control (filled circles) and MCK-CD36 (empty circles) mice (16–20 weeks old) were fasted for 4 h. Basal blood samples were taken prior (time 0) to tail injection of glucose (1 mg/kg) and at the indicated time intervals (5, 15, 30, 60, and 120 min) after the injection. Blood glucose was measured using a Precision Q.I.D. monitoring system. *p < 0.01, n = 6.
addition, since susceptibility to these effects is dependent on the mouse strain, strains other than FVB will also have to be examined (36). It is interesting to point out two lines of evidence that suggest a link between CD36 expression and insulin responsiveness. A causal genetic linkage between deficiency in CD36 and symptoms of insulin resistance was recently noted in SH rats (13). Also, many insulin-sensitizing drugs are peroxisome proliferator-activated receptor agonists and strong CD36 inducers (11, 37), and one of the earliest and most notable effects of these drugs in diabetic animals is a drop in blood FA (38).

The findings with MCK-CD36 mice are likely to be relevant to the human situation. CD36 deficiency is prevalent in 0.3–1% of the population. The most common cases involve one nucleotide (1159) insertion in codon 317, which leads to a frameshift and the appearance of a premature stop codon (17) and one amino acid substitution (Cys478 to Thr) (39). Previous reports documented an association between deficiency in CD36, defects of lipid metabolism and insulin resistance. For example, subjects with CD36 deficiency may have abnormalities in their ability to sustain endurance exercise. On the other hand, they might be more resistant to the negative effects of high fat diets such as obesity and atherosclerosis.

In summary, this study documented the role of CD36 in membrane FA uptake by muscle and the role of uptake in regulating muscle FA utilization. Alterations in these processes were shown to impact the metabolic profile and lean mass of the animal. Tissue-targeted manipulation of CD36 expression in mice may provide unique models to examine the link between FA utilization, obesity, atherosclerosis, and insulin responsiveness of glucose metabolism.

Acknowledgment—X. L. and K. Z. thank C. S. Landis and Drs. A. Wishnia and C. S. Springer for help and provision of the magnetic resonance imaging experiments. We are grateful to Drs. S. Levak-Frank and R. Zechnier for help in constructing the transgene MCK-CD36 and to C. Picken and Dr. T. Rosenquist for technical help in growing the transgenic animals.

REFERENCES
Muscle-specific Overexpression of FAT/CD36 Enhances Fatty Acid Oxidation by Contracting Muscle, Reduces Plasma Triglycerides and Fatty Acids, and Increases Plasma Glucose and Insulin

Azeddine Ibrahimi, Arend Bonen, W. Dennis Blinn, Tahar Hajri, Xin Li, Kai Zhong, Roger Cameron and Nada A. Abumrad

doi: 10.1074/jbc.274.38.26761

Access the most updated version of this article at http://www.jbc.org/content/274/38/26761

Alerts:

• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 38 references, 16 of which can be accessed free at http://www.jbc.org/content/274/38/26761.full.html#ref-list-1