methods

# FAT/CD36 expression is not ablated in spontaneously hypertensive rats

**Arend Bonen,[1,*] Xiao-Xia Han,[*] Narendra N. Tandon,[†] Jan F. C. Glatz,[§] James Lally,[*] Laelie A. Snook,[*] and Joost J. F. P. Luiken[§]**

Department of Human Health and Nutritional Sciences University of -Guelph,* Guelph, Ontario, N1G 2W1, Canada; Thrombosis Research Laboratory,† Otsuka Maryland Medicinal Laboratories 9900 Medical Center Drive, Rockville, MD 20850, and Department of Molecular Genetics,§ Maastricht University, 6200-MD Maastricht, The Netherlands

**Abstract There is doubt whether spontaneously hypertensive rats (SHR; North American strain) are null for fatty acid translocase (FAT/CD36). Therefore, we examined whether FAT/CD36 is expressed in heart, muscle, liver and adipose tissue in SHR. Insulin resistance was present in SHR skeletal muscle. We confirmed that SHR expressed aberrant FAT mRNAs in key metabolic tissues; namely, the major 2.9 kb transcript was not expressed, but 3.8 and 5.4 kb transcripts were present. Despite this, FAT/CD36 protein was expressed in all tissues, although there were tissue-specific reductions in FAT/CD36 protein expression and plasmalemmal content, ranging from 26–85%. Fatty acid transport was reduced in adipose tissue (−50%) and was increased in liver (+47%). Normal rates of fatty acid transport occurred in heart and muscle, possibly due to compensatory upregulation of plasmalemmal fatty acid binding protein (FABPpm) in red (+123%) and white muscle (+110%). In conclusion, SHRs (North American strain) are not a natural FAT/CD36 null model, the North American strain of SHR express FAT/CD36, albeit at reduced levels.—**Bonen, A., X-X. Han, N. N. Tandon, J. F. C. Glatz, J. Lally, L. A. Snook, and J. J. F. P. Luiken. **FAT/CD36 expression is not ablated in spontaneously hypertensive rats. *J. Lipid Res.* 2009.** 50: **740–748.**

**Supplementary key words** muscle • heart • liver adipose • tissue • glucose transport • fatty acid transport

Flux of long chain fatty acids (LCFA) across the plasma membrane, in adipose tissue, liver, heart, and skeletal muscle, occurs largely via a protein-mediated mechanism [cf (1–3)]. A number of fatty acid transport proteins have been identified including fatty acid translocase (FAT/CD36), plasma membrane associated fatty acid binding protein (FABPpm), and fatty acid transport protein 1 (FATP1) [cf (1, 4)]. Among these, FAT/CD36 is thought to be key, as in mammalian tissues its ablation (5–9) or inhibition (8, 10, 11) markedly reduces LCFA transport and metabolism, while its overexpression increases LCFA transport and metabolism (12–14).

Because insulin resistance is known to be associated with high concentrations of circulating fatty acids and intramuscular lipid accumulation, it has been of interest to examine the regulation of LCFA transport and transporters in obesity and type-2 diabetes. In FAT/CD36 null mice, insulin sensitivity is increased (15). In contrast, in insulin resistant animal models of obesity (16–18) and type 2 diabetes (19), as well as in human obesity and type 2 diabetes (20), the rates of LCFA transport are increased in skeletal muscle, heart, and adipose tissue (16–20). This increased LCFA influx was associated with an increase in plasmalemmal FAT/CD36 in muscle (16–18, 20), and in plasmalemmal FAT/C36 and FABPpm in heart and adipose tissue (16). However, except in severe, untreated type 2 diabetes (19), the protein expression of these fatty acid transporters was unaltered in insulin resistant animals and humans (16, 17, 20), indicating that these proteins can be relocated within heart, muscle, and adipose tissue (16, 17, 20). Taken altogether, there is strong evidence linking insulin resistance with increased rates of LCFA transport and increased plasmalemmal content of FAT/CD36 in metabolically important tissues in obesity and type 2 diabetes.

Spontaneously hypertensive rats (SHR) are a well-known model of insulin resistance (21, 22). However, in contrast

*These studies were supported in part by grants from the Canadian Institutes of Health Research (AB), the Natural Sciences and Engineering Research Council of Canada (AB), the Heart and Stroke Foundation of Ontario (AB), The Netherlands Heart Foundation grant 2002.T049 (JG), the Netherlands Organization for Health Research and Development (NWO-ZonMw grant 40-00812-98-03075) (JL and JG), and the European Commission (Integrated Project LSHM-CT-2004-005272, Exgenesis) (JL and JG), and the Canada Research Chair program (AB). J.J.F.P. Luiken was 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.*

*Manuscript received 8 May 2008 and in revised form 23 September 2008 and in re-revised form 5 December 2008.*

*Published, JLR Papers in Press, December 9, 2008.*
*DOI 10.1194/jlr.M800237-JLR200*

[1] To whom correspondence should be addressed.
e-mail: abonen@uoguelph.ca

to the reports linking increased FAT/CD36 to insulin resistance (16–20), there have been other reports (23–27) that have concluded that an FAT deletional mutation is at the root of insulin resistance in SHR sublines originating from the National Institutes of Health in North America. Specifically, in this SHR strain there was a differential hybridization signal for FAT mRNA and an apparent lack of any FAT/CD36 protein in adipocytes (23). This was accompanied by adipocyte insulin resistance and impaired catecholamine-stimulated fatty acid release, which was taken to be an index of plasmalemmal fatty acid transport (23). However, a subsequent study in the same SHR strain found that B-methlyiodophenylpentadecanoic acid uptake was only reduced in some tissues, namely heart (−25%) and adipose tissue (−60%), but not in liver or skeletal muscle (28). This may suggest that FAT/CD36 was not necessarily absent in these tissues, as had been reported originally (23). Indeed, close inspection of the data in SHR reveals a faint 2.8 kb transcript in the SHR heart (23), suggesting that some FAT/CD36 protein is expressed in SHR heart, and possibly in other nonadipose tissues. In addition, with aberrant FAT splicing, FAT/CD36 protein may still be formed (29). Others have shown that there appear to be no differences in lipid metabolism, or in basal and insulin-stimulated glucose transport in the SHR strains that harbored either the mutant FAT mRNA (SHR maintained in North America) or the normal FAT transcript (SHR maintained in Japan) (29). In WISTAR-KYOTO (WKY) and stroke-prone SHR animals, lipid metabolism differed, despite similar protein expression of adipocyte FAT/CD36 protein (30). Thus, questions have been raised as to whether *a*) the North American SHR strain are null for FAT/CD36, and *b*) whether a FAT/CD36 deficiency underlies insulin resistance in these animals.

We have examined in the North American WKY and SHR strains the expression of FAT/CD36 at the mRNA and protein expression level, as well at the plasma membrane, in metabolically important tissues (liver, adipose tissue, heart, and red and white skeletal muscle). We also examined the rates of fatty acid transport into giant vesicles prepared from all these tissues. To confirm that the North American SHR strain is insulin resistant, we determined the rates of insulin-stimulated glucose transport in skeletal muscle, a tissue that constitutes ∼40% of body mass and is the major site for insulin-stimulated glucose disposal. Although we confirm the presence of skeletal muscle insulin resistance and the FAT mutation in the SHR strain in all tissues examined, our data demonstrate that this SHR strain is not an FAT/CD36 null model, as these animals express FAT/CD36 protein, albeit at reduced levels, in heart, muscle, liver, and adipose tissue.

## METHODS

### Materials

[9,10-$^{3}$H]palmitate (American RadioChemicals St. Louis, MO), [$^{14}$C]mannitol, and [$^{3}$H] 3-O-methyl glucose (ICN, Oakville, Ont) were purchased from commercial sources. Collagenase type II was from Worthington Biochemical Co. (Lakewood, NJ), and collagenase VII was from Sigma-Aldrich (St. Louis, MO). Fat-free BSA was obtained from Roche Diagnostics (Laval, Quebec). The cDNAs For FAT/CD36 (31), FATP1 (32), and mitochondrial aspartate aminotransferase/FABPpm (33) were kindly provided by Dr. N.A. Abumrad (Washington University School of Medicine, St. Louis, MO), Dr. J. Schaffer (Washington University School of Medicine, St. Louis, MO), and Dr. A. Iriarte (University of Missouri, MO), respectively. For mRNA determinations selected materials were obtained from Promega BioSciences (San Luis Obispo, CA), Bio-Rad Laboratories (Mississauga, Ontario), and Boehringer-Ingelheim (Burlington, Ontario), as we have noted for the specific procedures (see later discussion). Antibodies against human CD36 (MO25) (34) and anti-FABPpm antiserum have been used in our previous work (16, 17, 19, 20, 35). We also used another antibody to detect FAT/CD36 (Abcam, Cambridge, MA), Similarly, a commercially available antibody was also used to detect FATP1 (Santa Cruz Biotechnology Inc., Santa Cruz, CA). All other reagents were from Sigma-Aldrich (St. Louis, MO).

### Animals

We used the same SHR strain that was used by Aitman et al. (23) and others (25, 26) in which they reported an apparent deletion of FAT/CD36. These hypertensive rats and their controls (WKY) were obtained from Charles River (Montreal, Quebec). At 3 months of age, animals were anesthetized with an intraperitoneal injection of Somnotol (6 mg/100 g weight). Subsequently, hindlimb muscles (red and white gastrocnemius), liver, epididymal adipose tissue, and heart were removed for fatty acid transport studies and for the determination of fatty acid transporters (FAT/CD36, FABPpm, FATP1) at the mRNA and protein expression level, and at the plasma membrane. In WKY and SHR subgroups of animals, we determined the rates of basal and insulin stimulated glucose transport in perfused hindlimb muscles.

### Isolation of giant vesicles and palmitate transport

Giant vesicles from heart, and red and white skeletal muscle were prepared as previously described (11, 35, 36). Minor modifications were introduced to prepare giant vesicles from liver and adipose tissue (16, 37). Briefly, all the tissues were cut into thin layers (1–3 mm thick) and incubated for 1 h at 34°C in 140 mM KCl-10 mM MOPS (pH 7.4), aprotinin (30 μg/ml), and collagenase in a shaking water bath. Collagenase type VII (150 U/ml) was used for skeletal muscle and liver tissues; collagenase type II (0.3%, wt/vol) was used for heart, and adipose tissue (0.05%, wt/vol). At the end of the incubation, the supernatant was collected, and the remaining tissue was washed with KCl-MOPS and 10 mM EDTA, which resulted in a second supernatant. Both supernatant fractions were pooled, and Percoll, KCl, and aprotinin were added to final concentrations of 3.5% (v/v), 28 mM, and 10 μg/ml, respectively. The resulting suspension was placed at the bottom of a density gradient consisting of a 3-ml middle layer of 4% Nycodenz (wt/vol) and a 1-ml KCl-MOPS upper layer. This sample was centrifuged at 60 *g* for 45 min at room temperature. Subsequently, the vesicles were harvested from the interface of the upper and middle layers, diluted in KCl$^{-}$ MOPS, and recentrifuged at 12, 000 *g* for 5 min. Respective red and white muscles from the same animal were pooled (i.e., pooled red muscles: red gastrocnemius, red vastus lateralis, and red tibialis anterior; pooled white muscles: white gastrocnemius, white vastus lateralis, and white tibialis anterior).

Palmitate uptake studies were performed as we have previously described (11, 16, 35–37). Briefly, 40 μl of 0.1% BSA in KCl-MOPS, containing unlabeled (15 μM) and radiolabeled 0.3 μCi [$^{3}$H]palmitate, and 0.06 μCi [$^{14}$C]mannitol, were added to 40 μl of

vesicle suspension. The incubation was carried out for 15 s. Palmitate uptake was terminated by addition of 1.4 ml of ice-cold KCl-MOPS, 2.5 mM HgCl2, and 0.1% BSA. The sample was then quickly centrifuged and the supernatant fraction was discarded. Thereafter, radioactivity was determined in the remaining pellet. Nonspecific uptake was measured by adding the stop solution before addition of the radiolabeled palmitate solution.

### Fatty acid transporters

*Northern blots.* The FAT mRNA was measured using previously described procedures (10, 16, 38, 39). Briefly, total RNA was isolated from tissues using the GIT/CsCl centrifugation method (40), with some modifications. The tissues were homogenized in 10 ml of 4M guanidine isothiocyanate, and layered on top of 3.3 ml of 5.7M cesium chloride solution. The samples were centrifuged in an SW-41 Ti rotor (Beckman), at 30K rpm for 23 h. The RNA pellets were recovered and purified by two precipitations in ethanol. For Northern analysis 5 μg of total RNA was used for electrophoresis on 1.2% formaldehyde agarose gels (41), and then transferred to positively charged nylon membrane (Boehringer-Ingelheim). The Northern blots were UV cross-linked with a GS-Gene linker (Bio-Rad).

FAT cDNA (31) was subcloned into *Eco*RI site of pBluescript (KS). The orientation was checked by digestion of template DNA with AccI restriction enzyme. DIG-labeled antisense riboprobe, 1.6 kb long, was generated by digestion of the template DNA with AseI restriction enzyme, and in vitro transcription with T3 RNA polymerase. The orientation was checked by digestion of template DNA with *Hin*dIII restriction enzyme. DIG-labeled antisense riboprobe was generated by in vitro transcription with T7 RNA polymerase after linearization of template DNA with XhoI restriction enzyme.

The ingredients for RNA transcription included 1–2 μg of DNA template plus the nucleotide triphosphate mix [2.5 mM rCTP, 2.5 mM rGTP, 2.5 mM rATP, 1.625 mM UTP (Promega) and 0.875 mM Dig-11 UTP (Boehringer Ingelheim)], 20 mM DTT (Promega), 1U / 1 μg template DNA of RNase Inhibitor (Promega), and 1× RNA polymerase buffer [5× buffer: 400 mM Tris-HCl pH 7.5; 60 mM MgCl2 and 20 mM spermidine-HCl (Promega)] maintained at room temperature. The appropriate RNA polymerase [T3 and T7 RNA polymerases (Boehringer-Ingelheim)] was added (at least 20 IU/1 μg of DNA template) and incubated for 2 h at 37°C. The DNA template was then digested for 10 min at 37°C with RNase-free Dnase (1 IU/1 μg of DNA template; Promega). After precipitation in ethanol and centrifugation at 12,000 rpm for 15 min, the probe was resuspended in 10–20 ml DIG Easy-Hyb hybridization buffer (Boehringer-Ingelheim) at concentrations of 50–100 ng/ml.

After prehybridization of the membrane for at least 4 h at 68°C, the DIG Easy-Hyb hybridization buffer was replaced with buffer containing DIG-labeled antisense RNA probe and membrane was incubated with the probe overnight at 68°C. Chemiluminescent detection was performed in accordance with the protocol supplied by the manufacturer (Boehringer Ingelheim), and the membrane was exposed to Kodak BioMax film. After exposure the film was developed in Kodak developer and fixed in Kodak fixer.

*Western blotting.* FAT/CD36 protein content was determined in both homogenates and in the plasma membrane of giant vesicles prepared from heart, muscle, adipose tissue, and liver as we have described previously (10, 16, 37, 39). The antibody against FAT/CD36 has been used in our previous studies (7, 20, 35). In addition, FAT/CD36 protein expression was also detected with a commercially available antibody (Abcam, Cambridge, MA). We also examined fatty acid transport protein levels in homogenates from muscle, heart, liver, and adipose tissue in Wistar rats, but because these were similar to those in WKY animals (data not shown) further studies in these rats were not performed.

Varying amounts of protein were loaded for each of the tissues and fractions studied. For whole tissue homogenates, 20 μg of adipose tissue protein, 30 μg of red muscle and white muscle protein, 15 μg heart tissue protein, and 40 μg of liver tissue protein were loaded. For sarcolemmal samples, 15 μg of red muscle and white muscle vesicle protein, 7.5 μg heart vesicle, 20 μg of adipose vesicle protein, and 25 μg of liver vesicle protein were loaded. The samples were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes. The membranes were then blocked for at least 1 h in BSA (7.5% in TBS-T at room temperature) and then incubated with the primary antibody for FAT/CD36 (MO25, 1:20,000 dilution; commercially available antibody (Abcam, Cambridge, MA), 1:1,000 dilution) overnight at 4C. After incubation with appropriate secondary antibodies (FAT/CD36 MO25 1:20,000 anti-mouse), the membranes were detected using enhanced chemiluminescence (Perkin Elmer, Waltham, MA). Blots were quantified with densitometry and corrected for loading using Ponceau staining (ChemiGenius 2 Bioimaging system; SynGene, Cambridge, UK).

### Glucose transport

To determine the insulin sensitivity of skeletal muscle basal and insulin-stimulated glucose transport rates were examined using a hindlimb perfusion procedure as we have previously described (42–46). For these purposes the animals were surgically prepared under anesthesia (65 mg pentobarbital sodium/100 g body wt). The cell-free perfusate consisted of a Krebs-Henseleit buffer, 2 mM pyruvate, 4% BSA under constant gassing (95% O2/5% CO2). Initially (5 min) the venous outflow was discarded and the perfusion flow was adjusted to 18 ml/min. Thereafter, muscles were preperfused, either without (basal) or with insulin (20 mU/ml) for 20 min. At that point 3-O-methylglucose, corresponding to concentrations at which the maximal rate of glucose transport occurs (40 mM, 10 μCi [$^3$H]3-O-MG), was added to the perfusion reservoir. Upon completion of the perfusion, the red and white gastrocnemius muscles were immediately excised, blotted for excess liquid on paper, and frozen in liquid $N_2$. In all experiments, mannitol (2 mM, 10 μCi [$^{14}$C]mannitol) was used as an extracellular space marker. Muscle samples were boiled with 1 N NaOH for 15 min and chilled on ice. Thereafter, 200 μl of aqueous solution as well as perfusate sample were counted in a liquid scintillation counter. Determinations of 3-O-MG transport rate were performed using standard calculations.

### Statistics

Differences in SHR and WKY rats were compared using analyses of variance and Fisher's least squares difference posthoc test, when appropriate. All data are reported as mean ± SEM.

## RESULTS

### Glucose transport in skeletal muscle

Because skeletal muscle comprises 40% of body mass and is a key tissue in which insulin resistance is commonly observed in SHR (21, 22), we examined the rates of 3-O-methyl glucose transport into perfused rat hindlimb muscles of SHR and WKY rats. Basal rates of 3-O-methyl glucose transport did not differ in WKY and SHR ($P > 0.05$, **Fig. 1A**). Insulin-stimulated rates of 3-O-methyl glucose transport were increased up to 7-fold in both WKY and SHR, depending on the muscle type (Fig. 1B). However, the insulin-

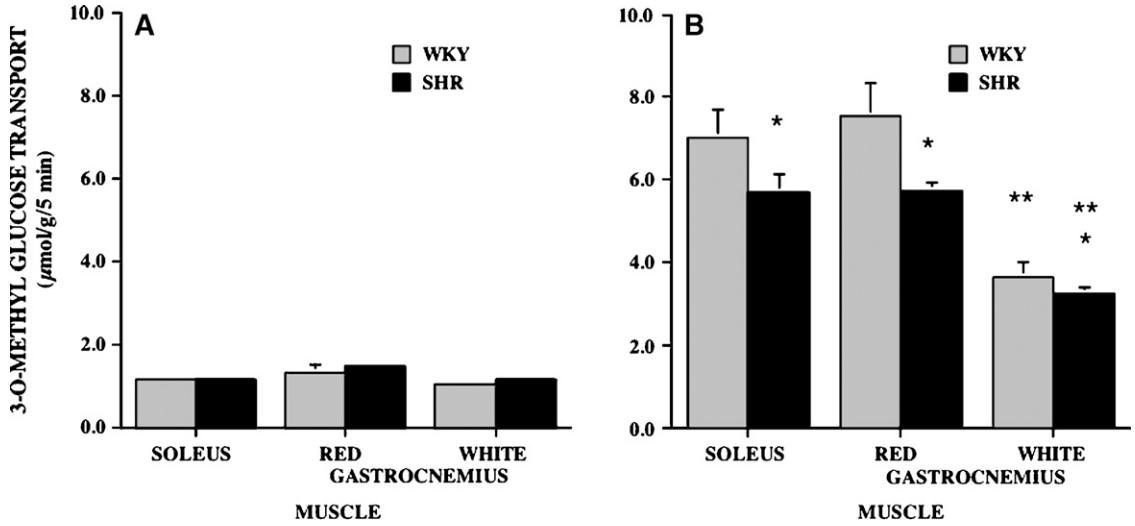


**Fig. 1.** Basal (A) and insulin stimulated glucose transport (B) in hindlimb perfused muscles in WKY and spontaneously hypertensive rats (SHR). Hindlimb perfusion was performed using controlled flow rates and radiolabeled 3-O-methylglucose with (20 mU/ml) or without insulin (basal) as described in the Methods. Data are mean ± SEM, N = 4 for each treatment * $P < 0.05$, SHR vs. WKY ** $P < 0.05$, white muscle vs. red muscle.

stimulated glucose transport rates were lower in SHR ($P < 0.05$: white gastrocnemius, −10%; soleus, −19%; red gastrocnemius, −24%) (Fig. 1B).

### Fatty acid transporters and fatty acid transport in muscle, heart, liver, and adipose tissue

In several studies it has been reported that FAT mRNA exhibited aberrant transcripts and that FAT/CD36 protein expression was not evident in adipose tissue when comparing SHR to WKY rats (23, 25, 26). Therefore, in key metabolic tissues, (liver, heart, red and white skeletal muscles, and adipose tissue) we examined FAT mRNA, FAT/CD36 protein expression, and FAT/CD36 plasma membrane content. The rates of fatty acid transport into giant vesicles prepared from muscle, heart, liver, and adipose tissue were also determined.

### mRNA expression of fatty acid transporters

A major FAT transcript is found in rodents at 2.8–2.9 kb (23, 31). In WKY rats the major FAT transcript was found at 2.9 kb (**Fig. 2A, B**). In the SHR, FAT mRNA showed transcript variants in all tissues examined. The 2.9 kb transcript was not detected in the SHR strain (Fig. 2A, B). Instead, FAT mRNA transcripts in the SHR were observed at 3.8kb (Fig. 2A, C) and 5.4 kb (Fig. 2A, D). In SHR there were only differences in FABPpm mRNA and FATP1 mRNA in selected tissues [FABPpm mRNA: white muscle −45%, liver −66%); FATP1 mRNA: adipose tissue +72%) (data not shown)].

### Protein expression of fatty acid transporters

In all tissues examined, the total FAT/CD36 protein pool in homogenates was lower in SHR than in WKY rats ($P < 0.05$, **Fig. 3A–C**). In heart, red and white muscles from SHR, FAT/CD36 protein contents were 26%, 40%, and 53% lower (Fig. 3A), respectively, while in adipose tissue (Fig. 3B) and liver (Fig. 3C), the reductions were 46% and 75%, respectively. To further confirm that FAT/CD36 protein was indeed expressed in SHR, we also examined the protein expression using a commercially available antibody. With this antibody, FAT/CD36 protein was also detected in all tissues examined in both WKY and SHR (see Fig. 3 inset). No differences between WKY and SHR were observed in total FABPpm and FATP1 protein expression in most instances, but there were exceptions [FABPpm: adipose tissue −35%; FATP1: heart −35% (data not shown)].

### Fatty acid transporters at the plasma membrane

Due to the fact that the functional pool of fatty acid transporters are located at the plasma membrane and because FAT/CD36 can be redistributed to the plasma membrane in skeletal muscle and heart, without altering FAT/CD36 protein expression (14, 16–18, 20), it was important to examine the plasmalemmal content of FAT/CD36. In all tissues examined, plasmalemmal FAT/CD36 was reduced in SHR ($P < 0.05$; **Fig. 4**). Specifically, these reductions were as follows: heart −36%, red muscle −56%, white skeletal muscle −71%, adipose tissue −59%, liver −85%. When using a commercially available antibody, reductions in SHR plasmalemmal FAT/CD were also evident (see Fig. 4 inset). In SHR, plasmalemmal FABPpm was only altered in red (+123%, $P < 0.05$) and white skeletal muscle (+110%), while hepatic plasmalemmal FATP1 was increased minimally, but consistently (+17%, $P < 0.05$) (data not shown).

### Fatty acid transport into giant vesicles in SHR and WKY rats

Rates of fatty acid transport were measured in giant vesicles prepared from heart, red and white muscles, liver, and adipose tissue. In WKY, fatty acid transport rates differed among the tissues (adipose tissue>heart>liver>red mus-

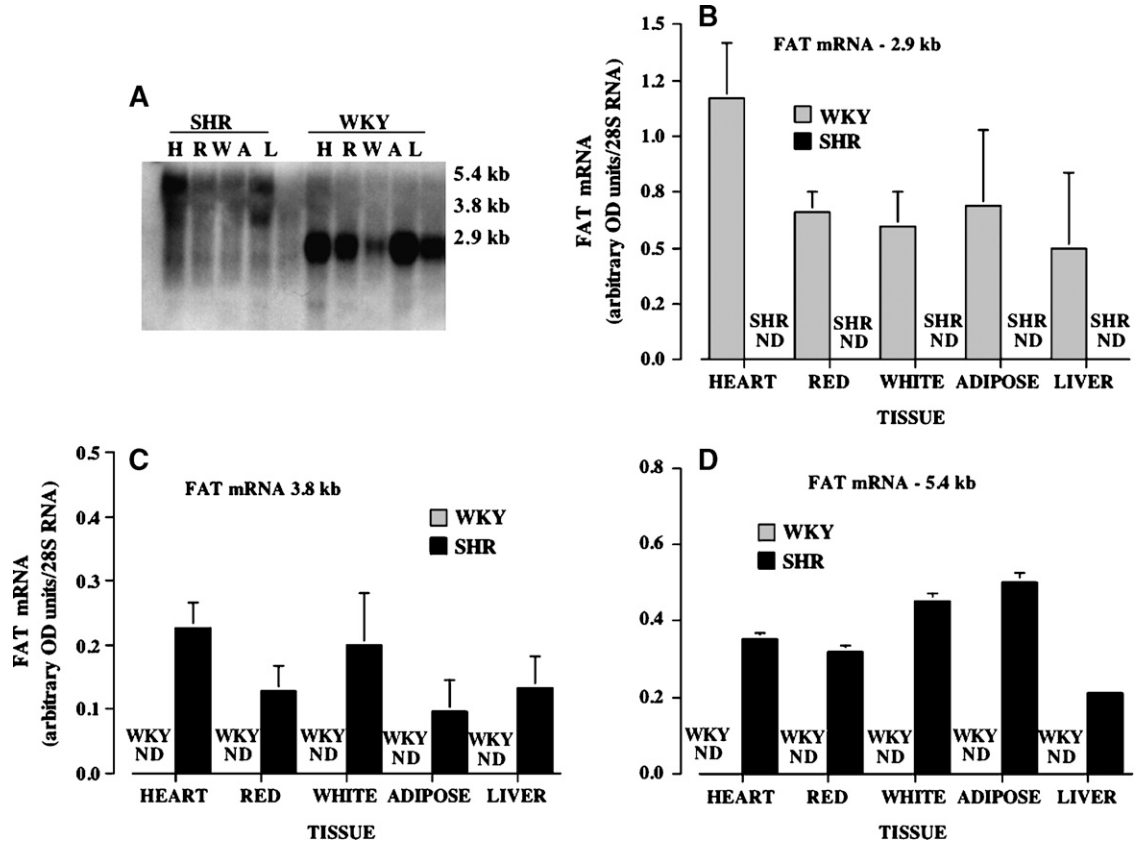


**Fig. 2.** FAT mRNA blot in heart (H), red (R) and white (W) muscle, adipose tissue (A), and liver (L) of WKY and SHR (A) and the quantification of 2.9 kb (B), 3.8 kb (C), and 5.4 kb (D) FAT transcripts in WKY and SHR. Data were quantified by determining the density of each blot, which was normalized for loading using 28S RNA. Data are mean ± SEM, N = 3–4 for each tissue examined in each group of animals SHR. ND, not detected.

cle>white muscle; **Fig. 5**). There was a 50% reduction in palmitate transport into adipose tissue from SHR compared with WKY ($P < 0.05$, Fig. 5). In contrast, no differences in palmitate transport rates were observed between WKY and SHR, in heart, or in red and white skeletal muscles ($P > 0.05$, Fig. 5), while in liver there was a 47% increase in the palmitate transport rate in SHR ($P < 0.05$, Fig. 5).

## DISCUSSION

We examined whether insulin resistant SHR were null for FAT/CD36, as had been proposed recently (23, 25, 26). Based on reduced rates of insulin-stimulated glucose transport into skeletal muscle, we confirmed that the SHR strain exhibits insulin resistance. Moreover, we also confirm that FAT mRNA transcripts differed in heart, muscle, liver, and adipose tissue of SHR when compared with WKY animals, as had been reported previously for adipose tissue (23, 29). This change in the predominant mRNA transcript in the SHR did not ablate FAT/CD36 protein expression. Instead, FAT/CD36 protein expression and plasmalemmal content in heart, muscle, liver, and adipose tissue were reduced by 26–85% in SHR. This was accompanied in SHR by tissue-specific changes in the mRNA and protein expression, and plasmalemmal content of other fatty acid transporters, which may compensate in part for the reductions in plasmalemmal FAT/CD36 in SHR.

### FAT mRNA and protein expression

Our observations of differing FAT transcripts in adipose tissue, heart, muscle, and liver of SHR and WKY rats concur with FAT transcripts observed in adipose tissue in other studies (23, 25, 29). It is now known that there can be aberrant FAT mRNA splicing in SHR strains obtained from different sources. Thus, in the original colony maintained in Japan both WKY and SHR expressed only the 2.8kb FAT/CD36 transcript, whereas in the SHR strain maintained in North America FAT/CD36 transcripts are present at 3.8 and 5.4 kb, but not at 2.8 kb (Refs. 23, 25, 29, and present study). Allele distribution patterns of chromosome four markers in the different SHR strains suggest that the FAT/CD36 mutation was introduced as a de novo mutation in SHR housed in North America but not those maintained Japan (29).

In several studies with the North American SHR strain (23, 25, 28), FAT/CD36 protein could not be detected in adipose tissue in which the 2.8 kb FAT mRNA was not present. Yet, despite observing the same FAT mRNA variants in the same SHR strain, we easily detected FAT/CD36 protein in adipose tissue as well as in all other tissues examined

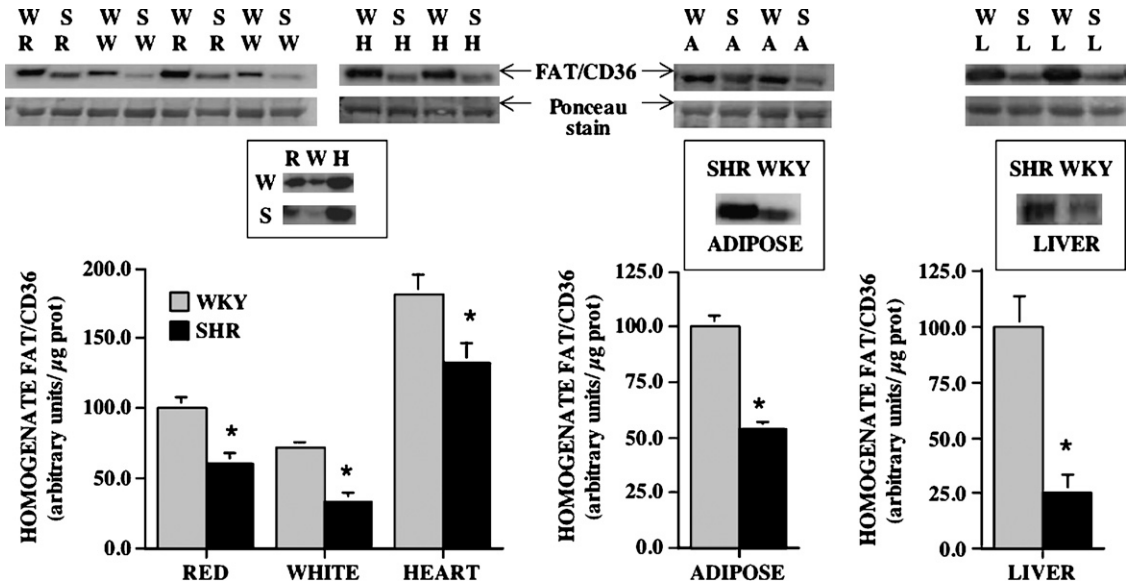


**Fig. 3.** Protein expression of fatty acid translocase (FAT/CD36) in heart, red and white skeletal muscle, adipose tissue, and liver in WKY and SHR. Data are mean ± SEM, N = 5–8 for each tissue examined in each group of animals. R, red muscle; W, white muscle; H, heart; A, adipose tissue; L, liver FAT/CD36 was detected at 88 kDa. Data were quantified by determining the density of each blot and normalized using Ponceau staining. Ponceau stains are shown below Western blot for FAT/CD36 * $P < 0.05$, SHR vs. WKY. Inset: FAT/CD36 protein expression in tissue homogenates using a commercially available antibody.

(heart, liver, and skeletal muscle). We have no obvious explanation for the discrepancies in FAT/CD36 protein expression observed in adipose tissue in our work and that of others (23, 25, 28). However, Aitman et al. (23) raised the possibility that the polyclonal antibody used in their work may not have detected the FAT/CD36 protein in adipose tissue in SHR (23).

Our observations that FAT/CD36 protein is indeed expressed in metabolically important tissues in SHR is bolstered by the fact that FAT/CD36 was detected whether we used the MO25 antibody or a commercially available antibody. There are also several other lines of evidence to indicate that the antibodies used in the present study are well suited to detect FAT/CD36 in mammalian tissues, as *a*) the FAT/CD36 protein expression followed the expected differences in muscle tissues in both SHR and WKY, as we have observed previously in other rat strains (10, 11, 16, 39); *b*) transfection of FAT into muscle upregu-

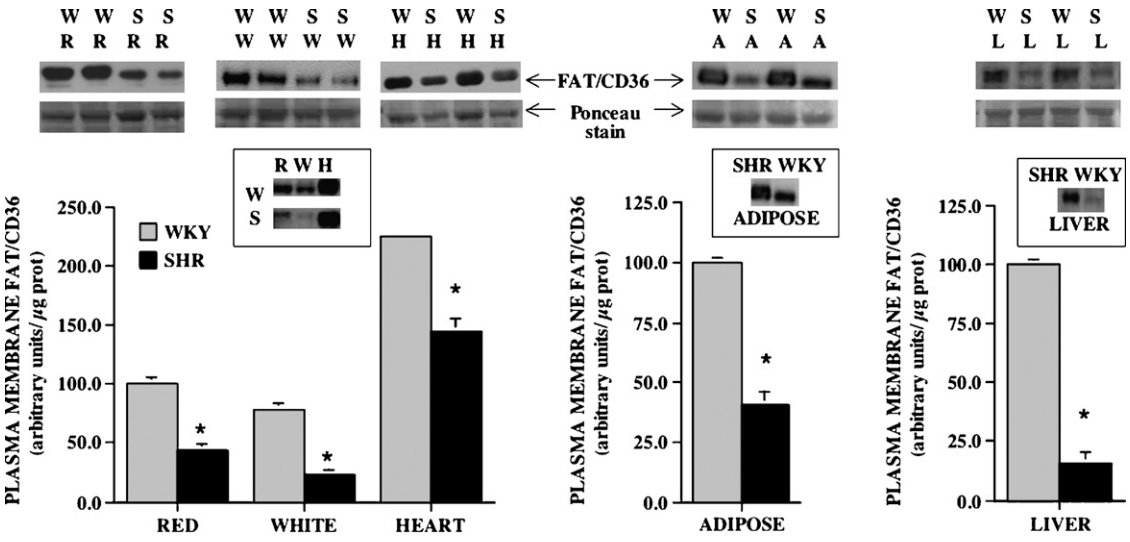


**Fig. 4.** Plasma membrane FAT/CD36 in heart, red and white skeletal muscle, adipose tissue, and liver in WKY and SHR. Data are mean ± SEM, N = 4–8 for each tissue examined in each group of animals. R, Red muscle; W, white muscle; H, heart; A, adipose tissue; L, liver FAT/CD36 was detected at 88 kDa. Data were quantified by determining the density of each blot and normalized using Ponceau staining. Ponceau stains are shown below Western blot for FAT/CD36 * $P < 0.05$, SHR vs. WKY. Inset: plasma membrane FAT/CD36 protein using a commercially available antibody.

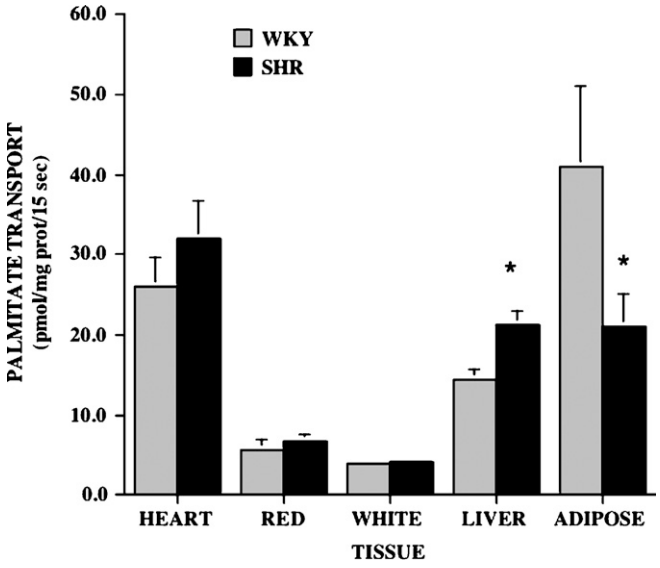


**Fig. 5.** Rate of palmitate transport into giant vesicles prepared from heart, red and white skeletal muscle, liver, and adipose tissue in WKY and SHR. Data are mean ± SEM, N = 6–8 for each tissue examined in each group of animals * $P < 0.05$, SHR vs. WKY.

lates FAT/CD36 protein expression (47); and *c*) upon *N*-linked deglycosylation of plasma membrane fractions, the molecular mass detected by the MO25 antibody (34) was reduced to 53 kDa (Luiken et al., unpublished data), in agreement with the reported molecular mass of native FAT/CD36 protein (31). Taken altogether, it appears that the antibodies used in our studies detect FAT/CD36 in a satisfactory manner. Therefore, we conclude that SHR are not null for FAT/CD36 protein.

### FAT/CD36 at the plasma membrane

Recent evidence has shown that FAT/CD36 has a key role in regulating the transmembrane flux of LCFAs and their metabolism [cf (1)]. Therefore, it was also important to examine not only FAT/CD36 protein expression, but also its plasmalemmal content. In the present study, the reduced FAT/CD36 protein expression also resulted in a reduced plasmalemmal FAT/CD36 content in all tissues in SHR when compared with WKY rats. The relative reduction (%) in FAT/CD36 protein expression among the tissues was highly correlated with the concurrent reduction at the plasma membrane ($r = 0.97$). This suggests that FAT/CD36 was not disproportionately redistributed to the plasma membrane to compensate for its reduced expression, nor would it seem that FAT/CD36 trafficking to the plasma membrane was impaired in SHR, as the reduction in plasmalemmal content reflected the reduction in expression.

### Palmitate transport into giant vesicles

It had been proposed that a reduced rate of adipose tissue glycerol release in the SHR strain provide functional evidence of FAT/CD36 ablation (23). However, rates of lipolysis would seem to be an inappropriate measure of fatty acid transport rates across the plasma membrane. Moreover, in other studies, impaired fatty acid uptake B-methlyiodophenylpentadecanoic acid was not observed in skeletal muscle of SHR (24). Therefore, we examined the rates of palmitate transport into giant vesicles. Giant vesicles have been thoroughly characterized as being fully suitable for determining rates of fatty acid transport (10, 11), and these vesicles have been used previously to measure rates of fatty acid transport in heart, muscle, liver, and adipose tissue (10, 11, 16, 37, 39). In the present study, the rates of palmitate transport were reduced only in giant vesicles prepared from adipose tissue, despite reductions in plasmalemmal FAT/CD36 in all tissues examined in the SHR strain. This was unexpected, as an increase or decrease in plasmalemmal FAT/CD36 is generally associated with concomitant changes in fatty acid transport [cf (1)]. This suggests that, except for adipose tissue, other fatty acid transport proteins may have compensated to maintain fatty acid transport in muscle, heart, and liver in the SHR strain.

Unaltered expression of FATP1 and plasmalemmal content in skeletal muscle cannot account for maintaining fatty acid transport in muscle, as this protein has a low fatty acid transport capacity compared with other FATPs (48) and FAT/CD36 (47). We have previously shown that an increase in plasmalemmal FABPpm can increase rates of palmitate transport (49, 50), and that this transporter can be induced to translocate from an intracellular depot to the plasma membrane (18, 51). Given that the transport capacity of FAT/CD36 is approximately 2-fold greater than that of FABPpm (47), it would seem that the doubling in plasmalemmal FABPpm in the present study compensated for the concomitant reductions of plasmalemmal FAT/CD36 in red (−56%) and white muscle (−71%), thereby maintaining the normal rates of palmitate transport.

Plasmalemmal FABPpm was not altered in SHR liver, but the increase in plasmalemmal FATP1 likely contributed to the increased rate of fatty acid transport in SHR liver. In heart, neither plasmalemmal FABPpm (unaltered) nor plasmalemmal FATP1 (decreased) can account for normal rate of fatty acid transport in SHR. In this tissue, the heart-specific transporter FATP6 (52) may have compensated to maintain rates of palmitate transport.

The large reduction in fatty acid transport into adipose tissue was attributable to the concurrent reduction in plasmalemmal FAT/CD36. In this tissue, neither plasmalemmal FABPpm nor plasmalemmal FATP1 were altered.

### Is FAT/CD36 associated with insulin resistance in SHR

It has been surmised that FAT/CD36 ablation was an underlying cause for diminished insulin-stimulated glucose uptake by adipocytes in SHR (23, 53). Yet, this linkage between FAT/CD36 deficiency and insulin resistance would not seem to be warranted. For example, the SHR strain that was thought to be FAT/CD36 null, exhibited either impaired oral glucose tolerance (24, 25) or no difference in oral glucose tolerance on either a chow (26) or a high-fat diet (24). Moreover, in FAT/CD36 null mice insulin sensitivity is improved (5, 15). Still others have shown that insulin resistance is present in other SHR strains in which FAT/CD36 is expressed normally (30). Thus, our present study and others (15, 29, 30) indicate that FAT/CD36 ablation/

reduction per sé is unlikely to be associated with insulin resistance in SHR. Instead, it is the increase in FAT/CD36 at the plasma membrane, in skeletal muscle and heart, that has been clearly associated with insulin resistance in animals (16–19, 54) and in humans (20), as this increases intracellular lipid accumulation.

## SUMMARY

The present studies have shown the North American SHR strain express aberrant FAT mRNAs in key metabolic tissues, which confirms previous reports in these animals. Despite these FAT mRNA anomalies, FAT/CD36 protein was expressed, albeit at reduced levels, the magnitude of which was tissue-specific. Reductions in FAT/CD36 protein and its plasmalemmal content in SHR strain resulted in a reduced rate of fatty acid transport into adipose tissue, but not in the other tissues examined. In skeletal muscle, maintenance of fatty acid transport rates was associated with a compensatory increase in plasmalemmal FABPpm. This was not observed in the other tissues. In liver, the increase in FATP1 may have contributed to the increase in fatty acid transport. We conclude that the North American SHR strain is not a naturally occurring FAT/CD36 null model.

## REFERENCES


1. Bonen, A., A. Chabowski, J. J. F. P. Luiken, and J. F. C. Glatz. 2007. Is membrane transport of FFA mediated by lipid, protein, or both? Mechanisms and regulation of protein-mediated cellular fatty acid uptake: molecular, biochemical, and physiological evidence. *Physiology (Bethesda).* **22:** 15–29.
2. Kampf, J. P., and A. M. Kleinfeld. 2007. Is membrane transport of FFA mediated by lipid, protein, or both? An unknown protein mediates free fatty acid transport across the adipocyte plasma membrane. *Physiology (Bethesda).* **22:** 7–14.
3. Kampf, J. P., A. M. Kleinfeld, A. Bonen, A. Chabowski, J. J. F. P. Luiken, and J. F. C. Glatz. 2007. Is membrane transport of FFA mediated by lipid, protein, or both?: Points of agreement. *Physiology (Bethesda).* **22:** 29.
4. Stahl, A. 2004. A current review of fatty acid transport proteins (SLC27). *Pflugers Arch.* **447:** 722–727.
5. Febbraio, M., N. A. Abumrad, D. P. Hajjar, K. Sharma, W. Cheng, S. Frieda, A. Pearce, and R. L. Silverstein. 1999. A null mutation in murine CD36 reveals an important role in fatty acid and lipoprotein metabolism. *J. Biol. Chem.* **274:** 19055–19062.
6. Coburn, C. T., F. F. Knapp, Jr., M. Febbraio, A. L. Beets, R. L. Silverstein, and N. A. Abumrad. 2000. Defective uptake and utilization of long chain fatty acids in muscle and adipose tissue of CD36 knockout mice. *J. Biol. Chem.* **275:** 32523–32529.
7. Bonen, A., X-X. Han, D. D. J. Habets, M. Febbraio, J. F. C. Glatz, and J. J. F. P. Luiken. 2007. 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.
8. Habets, D. D., W. A. Coumans, P. J. Voshol, M. A. den Boer, M. Febbraio, A. Bonen, J. F. Glatz, and J. J. Luiken. 2007. AMPK-mediated increase in myocardial long-chain fatty acid uptake critically depends on sarcolemmal CD36. *Biochem. Biophys. Res. Commun.* **355:** 204–210.
9. Kuang, M., M. Febbraio, C. Wagg, G. D. Lopaschuk, and J. R. Dyck. 2004. Fatty acid translocase/CD36 deficiency does not energetically or functionally compromise hearts before or after ischemia. *Circulation.* **109:** 1550–1557.
10. Bonen, A., J. J. Luiken, S. Lui, D. J. Dyck, B. Kiens, S. Kristiansen, L. Turcotte, G. J. van der Vusse, and J. F. Glatz. 1998. Palmitate transport and fatty acid transporters in red and white muscles. *Am. J. Physiol. Endocrinol. Metab.* **275:** E471–E478.
11. Luiken, J. J. F. P., L. P. Turcotte, and A. Bonen. 1999. Protein-mediated palmitate uptake and expression of fatty acid transport proteins in heart giant vesicles. *J. Lipid Res.* **40:** 1007–1016.
12. Bonen, A., D. J. Dyck, A. Ibrahimi, and N. A. Abumrad. 1999. Muscle contractile activity increases fatty acid metabolism and transport and FAT/CD36. *Am. J. Physiol.* **276:** E642–E649.
13. Ibrahimi, A., A. Bonen, W. D. Blinn, T. Hajri, X. Li, K. Zhong, R. Cameron, and N. A. Abumrad. 1999. 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.
14. Koonen, D., C. R. Benton, Y. Arumugam, N. N. Tandon, J. Calles-Escandon, J. F. Glatz, J. J. Luiken, and A. Bonen. 2004. Different mechanism can alter fatty acid transport when muscle contractile activity is chronically altered. *Am. J. Physiol. Endocrinol. Metab.* **286:** 1042–1049.
15. Goudriaan, J. R., V. E. Dahlmans, B. Teusink, D. M. Ouwens, M. Febbraio, J. A. Maassen, J. A. Romijn, L. M. Havekes, and P. J. Voshol. 2003. CD36 deficiency increases insulin sensitivity in muscle, but induces insulin resistance in the liver in mice. *J. Lipid Res.* **44:** 2270–2277.
16. Luiken, J. J. F. P., Y. Arumugam, D. J. Dyck, R. C. Bell, M. L. Pelsers, L. P. Turcotte, N. N. Tandon, J. F. C. Glatz, and A. Bonen. 2001. Increased rates of fatty acid uptake and plasmalemmal fatty acid transporters in obese Zucker rats. *J. Biol. Chem.* **276:** 40567–40573.
17. Coort, S. L. M., D. M. Hasselbaink, D. Y. P. Koonen, J. Willems, W. A. Coumans, A. Chabowski, G. J. van der Vusse, A. Bonen, J. F. C. Glatz, and J. J. F. P. Luiken. 2004. Enhanced sarcolemmal FAT/CD36 content and triacylglycerol storage in cardiac myocytes from obese Zucker rats. *Diabetes.* **53:** 1655–1663.
18. Han, X. X., A. Chabowski, N. N. Tandon, J. Calles-Escandon, J. F. Glatz, J. J. Luiken, and A. Bonen. 2007. Metabolic challenges reveal impaired fatty acid metabolism and translocation of FAT/CD36 but not FABPpm in obese Zucker rat muscle. *Am. J. Physiol. Endocrinol. Metab.* **293:** E566–E575.
19. Chabowski, A., J. C. Chatham, N. N. Tandon, J. Calles-Escandon, J. F. C. Glatz, J. J. F. P. Luiken, and A. Bonen. 2006. Fatty acid transport and FAT/CD36 are increased in red but not in white muscle skeletal muscle of Zucker diabetic fatty (ZDF) rats. *Am. J. Physiol. Endocrinol. Metab.* **291:** E675–E682.
20. Bonen, A., M. L. Parolin, G. R. Steinberg, J. Calles-Escandon, N. N. Tandon, J. F. C. Glatz, J. J. F. P. Luiken, G. J. F. Heigenhauser, and D. J. Dyck. 2004. 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.
21. James, D. J., F. Cairns, I. P. Salt, G. J. Murphy, A. F. Dominiczak, J. M. Connell, and G. W. Gould. 2001. Skeletal muscle of stroke-prone spontaneously hypertensive rats exhibits reduced insulin-stimulated glucose transport and elevated levels of caveolin and flotillin. *Diabetes.* **50:** 2148–2156.
22. Zecchin, H. G., R. M. Bezerra, J. B. Carvalheira, M. A. Carvalho-Filho, K. Metze, K. G. Franchini, and M. J. Saad. 2003. Insulin signalling pathways in aorta and muscle from two animal models of insulin resistance–the obese middle-aged and the spontaneously hypertensive rats. *Diabetologia.* **46:** 479–491.
23. Aitman, T. J., A. M. Glazier, C. A. Wallace, L. D. Cooper, P. J. Norsworthy, F. N. Wahid, K. M. Al-Majali, P. M. Trembling, C. J. Mann, C. C. Shoulders, et al. 1999. Identification of *Cd36 (Fat)* as an insulin-resistance gene causing defective fatty acid and glucose metabolism in hypertensive rats. *Nat. Genet.* **21:** 76–83.
24. Hajri, T., A. Ibrahimi, C. T. Coburn, F. F. Knapp, Jr., T. Kurtz, M. Pravenec, and N. A. Abumrad. 2001. Defective fatty acid uptake in the spontaneously hypertensive rat is a primary determinant of altered glucose metabolism, hyperinsulinemia, and myocardial hypertrophy. *J. Biol. Chem.* **276:** 23661–23666.
25. Pravenec, M., V. Landa, V. Zidek, M. Musilova, V. Kren, L. Kazdova, T. J. Aitman, A. Glazier, A. Ibrahimi, N. A. Abumrad, et al. 2001. Transgenic rescue of defective Cd36 ameliorates insulin resistance in spontaneously hypertensive rats. *Nat. Genet.* **27:** 156–158.
26. Qi, N., L. Kazdova, V. Zidek, V. Landa, V. Kren, H. A. Pershadsingh, E. S. Lezin, N. A. Abumrad, M. Pravenec, and T. W. Kurtz. 2002. Pharmacogenetic evidence that cd36 is a key determinant of the metabolic effects of pioglitazone. *J. Biol. Chem.* **277:** 48501–48507.
27. Pravenec, M., V. Landa, V. Zidek, A. Musilova, L. Kazdova, N. Qi,

J. Wang, E. St Lezin, and T. W. Kurtz. 2003. Transgenic expression of CD36 in the spontaneously hypertensive rat is associated with amelioration of metabolic disturbances but has no effect on hypertension. *Physiol. Res.* **52:** 681–688.
28. Hajri, T., X. X. Han, A. Bonen, and N. A. Abumrad. 2002. Defective fatty acid uptake modulates insulin responsiveness and metabolic responses to diet in CD36-null mice. *J. Clin. Invest.* **109:** 1381–1389.
29. Gotoda, T., Y. Lituzaki, N. Kato, J. Osuga, M-T. Bihoreau, T. Murakami, Y. Yamori, H. Shimano, S. Ishibashi, and N. Ymada. 1999. Absence of Cd36 mutation in the original spontaneously hypertensive rats with insulin resistance. *Nat. Genet.* **22:** 226–228.
30. Collison, M., A. M. Glazier, D. Graham, J. J. Morton, M. H. Dominiczak, T. J. Aitman, J. M. Connell, G. W. Gould, and A. F. Dominiczak. 2000. Cd36 and molecular mechanisms of insulin resistance in the stroke-prone spontaneously hypertensive rat. *Diabetes.* **49:** 2222–2226.
31. Abumrad, N. A., M. R. El-Maghrabi, E-Z. Amri, E. Lopez, and P. Grimaldi. 1993. Cloning of a rat adipocyte membrane protein implicated in binding or transport of long chain fatty acids that is induced during preadipocyte differentiation. Homology with human CD36. *J. Biol. Chem.* **268:** 17665–17668.
32. Schaffer, J. E., and H. F. Lodish. 1994. Expression cloning and characterization of a novel adipocyte long chain fatty acid transport protein. *Cell.* **79:** 427–436.
33. Mattingly, J. R., Jr., F. J. Rodriguez-Berrocal, J. Gordon, A. Iriarte, and M. Martinez-Carrion. 1987. Molecular cloning and in vivo expression of a precursor to rat mitochondrial aspartate aminotransferase. *Biochem. Biophys. Res. Commun.* **149:** 859–865.
34. Matsuno, K., M. Diaz-Ricard, R. R. Montgomery, T. Aster, G. A. Jamieson, and N. N. Tandon. 1996. Inhibition of platelet adhesion to collagen by monoclonal anti-CD36 antibodies. *Br. J. Haematol.* **92:** 960–967.
35. Bonen, A., J. J. F. P. Luiken, Y. Arumugam, J. F. C. Glatz, and N. N. Tandon. 2000. Acute regulation of fatty acid uptake involves the cellular redistribution of fatty acid translocase. *J. Biol. Chem.* **275:** 14501–14508.
36. Bonen, A., D. J. Dyck, and J. J. F. P. Luiken. 1998. Skeletal muscle fatty acid transport and transporters. *Adv. Exp. Med. Biol.* **441:** 193–205.
37. Koonen, D. P. Y., W. A. Coumans, Y. Arumugam, A. Bonen, J. F. C. Glatz, and J. J. F. P. Luiken. 2002. Giant membrane vesicles as a model to study cellular substrate uptake dissected from metabolism. *Mol. Cell. Biochem.* **239:** 121–130.
38. Bonen, A., D. Miskovic, and B. Kiens. 1999. Fatty acid transporters (FABPpm, FAT, FATP) in human muscle. *Can. J. Appl. Physiol.* **24:** 515–523.
39. Luiken, J. J., Y. Arumugam, R. C. Bell, J. Calles-Escandon, N. N. Tandon, J. F. Glatz, and A. Bonen. 2002. Changes in fatty acid transport and transporters are related to the severity of insulin deficiency. *Am. J. Physiol. Endocrinol. Metab.* **282:** 612–621.
40. Chirgwin, J., A. Przbyla, R. MacDonald, and W. Rutter. 1979. Isolation of biologically active ribonucleotic acid from source enriched in ribonuclease. *Biochemistry.* **18:** 5294–5299.
41. Sambrook, J., E. F. Fritisch, and T. Maniatis. *Molecular cloning: A laboratory manual*, Cold Spring Harbour Laboratory, Cold Spring Harbour NY, 1989.
42. Han, X. X., and A. Bonen. 1998. Epinephrine translocates GLUT-4 but inhibits insulin-stimulated glucose transport in rat muscle. *Am. J. Physiol.* **274:** E700–E707.
43. Lemieux, K., X-X. Han, L. Dombrowski, A. Bonen, and A. Marette. 2000. The transferrin receptor defines two distinct contraction-responsive GLUT4 vesicle populations. *Diabetes.* **49:** 183–189.
44. Han, X-X., P. Fernando, and A. Bonen. 2000. Denervation provokes greater reductions in insulin-stimulated glucose transport in muscle than severe diabetes. *Mol. Cell. Biochem.* **210:** 81–89.
45. Luiken, J. J. F. P., D. J. Dyck, X-X. Han, N. N. Tandon, Y. Arumugam, J. F. C. Glatz, and A. Bonen. 2002. Insulin induces the translocation of the fatty acid transporter FAT/CD36 to the plasma membrane. *Am. J. Physiol. Endocrinol. Metab.* **282:** E491–E495.
46. Benton, C. R., J. Nickerson, J. Lally, X-X. Han, G. P. Holloway, J. F. C. Glatz, J. J. F. P. Luiken, T. E. Graham, J. J. Heikkila, and A. Bonen. 2008. Modest PGC-1a overexpression in muscle in vivo is sufficient to increase insulin sensitivity and palmitate oxidation in SS, not IMF, mitochondria. *J. Biol. Chem.* **283:** 4228–4240.
47. Nickerson, J., and A. Bonen. 2005. Defining a role for skeletal muscle fatty acid transport proteins. In *2nd Northern Lights Conference. Canadian Federation of Biological Societies.* Guelph, Ontario, Canada. F47.
48. DiRusso, C. C., H. Li, D. Darwis, P. A. Watkins, J. Berger, and P. N. Black. 2005. Comparative biochemical studies of the murine fatty acid transport proteins (FATP) expressed in yeast. *J. Biol. Chem.* **280:** 16829–16837.
49. Clarke, D. C., D. Miskovic, X-X. Han, J. Calles-Escandon, J. F. C. Glatz, J. J. F. P. Luiken, J. J. Heikkila, and A. Bonen. 2004. Overexpression of membrane associated fatty acid binding protein (FABPpm) *in vivo* increases fatty acid sarcolemmal transport and metabolism. *Physiol. Genomics.* **17:** 31–37.
50. Holloway, G. P., J. Lally, J. G. Nickerson, H. Alkhateeb, L. A. Snook, G. J. Heigenhauser, J. Calles-Escandon, J. F. Glatz, J. J. Luiken, L. L. Spriet, et al. 2007. Fatty acid binding protein facilitates sarcolemmal fatty acid transport but not mitochondrial oxidation in rat and human skeletal muscle. *J. Physiol.* **582:** 393–405.
51. Chabowski, A., S. L. M. Coort, J. Calles-Escandon, N. N. Tandon, J. F. C. Glatz, J. J. F. P. Luiken, and A. Bonen. 2005. The subcellular compartmentation of fatty acid transporters is regulated differently by insulin and by AICAR. *FEBS Lett.* **579:** 2428–2432.
52. Gimeno, R. E., A. M. Ortegon, S. Patel, S. Punreddy, P. Ge, Y. Sun, H. F. Lodish, and A. Stahl. 2003. Characterization of a heart-specific fatty acid transport protein. *J. Biol. Chem.* **278:** 16039–16044.
53. Miyaoka, K., T. Kuwasako, K. Hirano, S. Nozaki, S. Yamashita, and Y. Matsuzawa. 2001. CD36 deficiency associated with insulin resistance. *Lancet.* **357:** 686–687.
54. Smith, A. C., K. L. Mullen, K. A. Junkin, J. Nickerson, A. Chabowski, A. Bonen, and D. J. Dyck. 2007. Metformin and exercise reduce muscle FAT/CD36 and lipid accumulation and blunt the progression of high-fat diet induced hyperglycemia. *Am. J. Physiol. Endocrinol. Metab.* **293:** E172–E181.