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# Overexpression of membrane-associated fatty acid binding protein (FABPpm) in vivo increases fatty acid sarcolemmal transport and metabolism

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**Clarke, David C., Dragana Miskovic, Xiao-Xia Han, Jorge Calles-Escandon, Jan F. C. Glatz, Joost J. F. P. Luiken, John J. Heikkila, and Arend Bonen.** Overexpression of membrane-associated fatty acid binding protein (FABPpm) in vivo increases fatty acid sarcolemmal transport and metabolism. *Physiol Genomics* 17: 31–37, 2004. First published December 23, 2003; 10.1152/physiolgenomics.00190.2003.—Fatty acid translocase (FAT/CD36) is a key fatty acid transporter in skeletal muscle. However, the effects on fatty acid transport by another putative fatty acid transporter, plasma membrane-associated fatty acid binding protein (FABPpm), have not been determined in mammalian tissue. We examined the functional effects of overexpressing FABPpm on the rates of *1*) palmitate transport across the sarcolemma and *2*) palmitate metabolism in skeletal muscle. One muscle (soleus) was transfected with pTracer containing FABPpm cDNA. The contralateral muscle served as control. After injecting the FABPpm cDNA, muscles were electroporated. FABPpm overexpression was directly related to the quantity of DNA administered. Electrotransfection (200 μg/muscle) rapidly induced FABPpm protein overexpression (*day 1*, +92%, $P < 0.05$), which was further increased during the next few days (*days 3–7*; range +142% to +160%, $P < 0.05$). Sarcolemmal FABPpm was comparably increased (*day 7*, +173%, $P < 0.05$). Neither FAT/CD36 expression nor sarcolemmal FAT/CD36 content was altered. FABPpm overexpression increased the rates of palmitate transport (+79%, $P < 0.05$). Rates of palmitate incorporation into phospholipids were also increased +36%, as were the rates of palmitate oxidation (+20%). Rates of palmitate incorporation into triacylglycerol depots were not altered. These studies demonstrate that in mammalian tissue FABPpm overexpression increased the rates of palmitate transport across the sarcolemma, an effect that is independent of any changes in FAT/CD36. However, since the overexpression of plasmalemmal FABPpm (+173%) exceeded the effects on the rates of palmitate transport and metabolism, it appears that the overexpression of FABPpm alone is not sufficient to induce completely parallel increments in palmitate transport and metabolism. This suggests that other mechanisms are required to realize the full potential offered by FABPpm overexpression.

muscle; giant vesicles; FAT/CD36; palmitate esterification; palmitate oxidation

FATTY ACID UPTAKE into metabolically important tissues occurs, in part, via a protein-mediated transport system (for review see Refs. 5, 37). A number of long-chain fatty acid transporters have been identified, including fatty acid transport proteins 1–5 (19, 38), fatty acid translocase (FAT/CD36) (1), and plasma membrane-associated fatty acid binding protein (FABPpm) (22, 42). Studies in our laboratory (7, 6, 20, 31, 27, 28) and others (10, 16) have established that FAT/CD36 regulates fatty acid uptake into muscle, heart, and adipose tissue. Moreover, we have shown that the insulin- and contraction-induced translocation of FAT/CD36 from intracellular depots to the plasma membrane increases palmitate uptake into muscle and heart (6, 29, 30). Overexpression of FATP1 (38) and FABPpm in 3T3 fibroblasts (22) can increase long-chain fatty acid uptake into these cell lines, but evidence for comparable actions by these two proteins in metabolically important mammalian tissues is presently lacking.

FABPpm is a 43-kDa peripheral membrane protein that is identical to mitochondrial aspartate aminotransferase (mAspAT) (2, 8, 43). This protein is localized to the mitochondrion and the plasma membrane (9). 3T3 fibroblasts transfected with a cDNA for mAspAT express FABPpm and exhibit saturable fatty acid uptake (22). FABPpm is expressed in most mammalian tissues examined. But, FABPpm is more abundantly expressed in heart compared with skeletal muscle (7, 31), and this may indicate that FABPpm expression is closely associated with the differing requirements for fatty acid metabolism in these tissues (heart $\gg$ muscle). Although FABPpm expression is enhanced when the oxidative capacity of muscle is increased by exercise training (23), it is not known whether this FABPpm increase results in greater rates of fatty acid transport and metabolism. We have observed that changes in long-chain fatty acid uptake into skeletal muscle occur either when parallel changes occur in plasmalemmal FAT/CD36 and FABPpm (27, 41), or when only plasmalemmal FAT/CD36, but not plasmalemmal FABPpm, is upregulated (28). To date, there have been no reports in which rates of fatty acid transport have been altered in mammalian tissue when only FABPpm, but not FAT/CD36, is upregulated. This may indicate that FABPpm, while being a necessary component of the sarcolemmal fatty acid transport system (31, 45), may play only a cooperative role with other fatty acid transporters. Therefore, we hypothesized that increasing the expression of plasmalemmal FABPpm, in the absence of corresponding changes in FAT/CD36, would not increase the rate of palmitate transport into muscle.

In the present study, we have examined the role of FABPpm on palmitate transport, independent of FAT/CD36. For these purposes we transfected, in vivo, a single skeletal muscle (soleus) of mature laboratory rats with a plasmid vector con-

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taining the FABPpm gene. Subsequently, we determined the effects of FABPpm protein overexpression on *1*) the rates of palmitate transport into giant sarcolemmal vesicles and *2*) on the rates of palmitate metabolism in intact skeletal muscle. The electrotransfection approach in the present study has several advantages, *1*) protein overexpression is achieved rapidly, in vivo, in fully mature skeletal muscle, a metabolically important tissue, and *2*) importantly, the contralateral soleus muscle in the same animal serves as control, and therefore many other possible confounding factors are controlled (e.g., substrate-endocrine milieu, dietary intake).

## METHODS

Male Sprague-Dawley rats (~300 g) were used for all experiments. Animals were housed in a climate and temperature controlled room on a 12:12-h reverse light-dark cycle. Rat chow and water were available ad libitum. The FABPpm gene was overexpressed in mature rat soleus muscle by electroporation of the muscle (see below). Studies were performed to examine the effects of different amounts of FABPpm cDNA (100, 200, 400 μg/muscle) on FABPpm protein expression, the time course of overexpression (1, 3, and 7 days), and the plasmalemmal localization of FABPpm. In addition, we examined the effects of overexpressing FABPpm on the rates of palmitate transport into giant sarcolemmal vesicles and on the rate of palmitate metabolism in intact soleus muscles (see below). All experimental procedures were approved by the Committee on Animal Care at the University of Waterloo.

*Subcloning and plasmid DNA preparation.* Two types of plasmid DNA were used in the electrotransfection experiments, pTracer-CMV2 (6.2 kb, Invitrogen) and pTracer-FABPpm (7.5 kb). Plasmid DNA was isolated using alkaline lysis and purified by polyethylene glycol (PEG) precipitation (36). FABPpm cDNA in pBluescript KS (pBluescript-FABPpm) was kindly provided by Dr. A. Iriarte (University of Missouri). The FABPpm cDNA was originally derived from mAspAT precursor mRNA (33). Using the pBluescript-FABPpm as a template, polymerase chain reaction (PCR) was performed using 5′-CCGTCTTACCAGCCACCATGCCCTCC forward and 5′-CCTGGTGATTACTTGGTGACCTGG reverse primers and *Taq* polymerase. The 27-nucleotide forward primer corresponded to nucleotide number 72–99 of the published sequence (GenBank accession no. M18467) and was designed to incorporate the Kozak sequence. The 24-nucleotide reverse primer corresponded to nucleotide number 1390–1367 of the published sequence. The resulting 1.3-kb PCR product was ligated into the *Eco*RV restriction enzyme site of pTracer, after the addition of terminal thymidine monophosphate residues to the 3′-ends of the linearized pTracer (32). Competent DH5a *Escherichia coli* cells were transformed using a calcium chloride protocol and plated on agar plates. The cloned PCR product was verified by DNA sequencing (Core Sequencing Facility, York University, Toronto).

*Electrotransfection of soleus muscle.* Animals were anesthetized with Somnotol (intraperitoneal 6.5 mg/100 g body mass; MTC Pharmaceuticals, Cambridge, ON) and provided with an analgesic (Temgesic, subcutaneous 30 mg/100 g body mass; Reckitt and Colman Pharmaceuticals). Thereafter, the shaved, lower hindlimb was sterilized (iodine and 70% ethanol), and electrode conductivity gel was applied to the medial aspect of the hindlimb. The entire ventral surface of the soleus was freed while not disrupting the connective tissue that encapsulates the soleus. Plasmid DNA was injected using a short (1.25 cm) 27-gauge needle. The needle was initially inserted into the distal aspect of the belly of the muscle and was directed proximally, parallel to the orientation of the muscle fibers. Upon injection, the area around the needle tip would swell with fluid. The needle was then pushed further proximal into the muscle and injection continued. This was repeated until the DNA had been delivered. To establish the relationship between FABPpm overexpression, the concentration of DNA injected was varied (0.5, 1.0, 2.0 μg/μl). Subsequently, all experiments were conducted using a DNA concentration of 1.0 μg/μl. For experimental control, the plasmid pTracer, devoid of FABPpm cDNA, was also electrotransfected into soleus muscles. For all experiments a total volume of 200 μl was injected into the soleus muscle.

Immediately following the DNA injection, a pair of 0.8-cm diameter plate electrodes, attached to a set of ruled calipers (BTX, San Diego, CA), were applied over the soleus muscle. The other electrode was applied to the underside of the medial aspect of the hindlimb. Electroporation of the intact soleus muscle was performed as described by Mir et al. (34) (8 electric pulses: 200 V/cm, 1 Hz, 20 ms in duration) (ECM 830 Square Wave Electroporator; BTX). After the pulse delivery, the overlying superficial muscle was sutured, and the skin incision was closed. Muscle tissue was sampled either 1, 3, or 7 days after transfecting the tissue. Unless otherwise noted, muscles were rapidly frozen using precooled tongs and stored at −80°C.

*Giant sarcolemmal vesicle preparation palmitate transport.* Giant vesicles were prepared from rat soleus muscle as we have described in detail elsewhere (6, 7, 27, 28, 31). For these purposes three to four soleii were pooled to obtain sufficient material for the palmitate transport studies and for Western blotting of the plasma membrane fractions of the vesicles. Palmitate transport into giant vesicles was determined as we have previously reported (6, 7, 27, 28, 31). Briefly, unlabeled palmitate (Sigma-Aldrich, Mississauga, Ontario, Canada) and [$^3$H]palmitate (0.3 μCi; Amersham Life Science, Oakville, Ontario) and [$^{14}$C]mannitol (0.06 μCi, Amersham Life Science) in a 0.1% BSA KCl/MOPS solution were added to 40 μl of vesicles (~80 μg protein). The reaction was carried out at room temperature for 15 s. Palmitate uptake was terminated by addition of 1.4 ml ice-cold KCl/MOPS, containing 2.5 mM $HgCl_2$ and 0.1% BSA. The sample was quickly centrifuged at maximal speed in a microcentrifuge for 1 min. The supernatant was discarded, and radioactivity was determined in the tip of the tube. Nonspecific uptake was measured by adding the stop solution to the membrane before the addition of the isotopes.

*Palmitate metabolism in isolated soleus muscles.* In control and transfected muscles we examined the metabolism of palmitate. For these studies we used procedures that we have reported previously (11, 12, 13, 24). Briefly, soleus muscle strips were preincubated (30°C) for 20 min, followed by a 60-min incubation period (30°C). The incubation medium contained 0.5 mM palmitate and 5 mM glucose, as well as 2 μCi of [1-$^{14}$C]palmitate (Amersham Life Science). A layer of mineral oil prevented the escape of $CO_2$ from the incubation buffer. Palmitate oxidation was determined by acidifying 0.5 ml of incubation buffer with 1.0 ml of 1 M $H_2SO_4$ in a sealed glass vial holding a suspended center well containing benzethonium hydroxide to trap the $CO_2$. To determine the radiolabeled palmitate incorporation into intramuscular lipid pools, we used procedures that we (12) have previously described. Muscles were blotted and weighed, and homogenized in a tissue homogenizer in 1.5 ml of ice-cold 1:2 chloroform:methanol solution. Thereafter, an additional 0.5 ml of pure chloroform was added and samples were again homogenized. Connective tissue was removed, blotted, and weighed, then subtracted from the muscle wet weight. Muscle samples were then spotted on silica gel plates (Silica Gel GF, 250 mm; Analtech, Newark, DE), and resolved in solvent (60:40:4, heptane-isopropylether-acetic acid) for 45 min. Plates were air-dried, sprayed with dichlorofluorescein dye (0.02% wt/vol in ethanol), and visualized under long-wave ultraviolet light. Individual lipid bands (phospholipids, triacylglycerols) were identified against known standards and marked on the plate with a scalpel and scraped into vials for liquid scintillation counting.

*Western and Northern blotting.* Proteins were detected, after separation by SDS-PAGE, using Western blotting procedures that we have described in detail elsewhere (6, 7, 27, 28, 31). The FABPpm polyclonal antibody was produced in one our laboratories (J. Calles-

Escandon) and has been used previously in our work elsewhere (27, 28, 31). The FAT/CD36 monoclonal antibody used in this study was a gift from Dr. N. N. Tandon (Otsuka America Pharmaceutical, Rockville, MD). MCT1 polyclonal antibody was a gift from Dr. H. Hatta (University of Tokyo), and caveolin-3 (Cav-3) and green fluorescent protein (GFP) monoclonal antibodies were obtained from BD Biosciences Clontech (Palo Alto, CA). The content of cytoplasmic heart-type cytosolic fatty acid binding protein (H-FABPc) in soleus was determined by a sandwich-type ELISA as previously described (47). RNA isolation and FABPpm mRNA detection using digoxigenin-labeled antisense riboprobes in Northern blot analysis as performed in our laboratory have been described in detail (27, 28).

*Biochemical assays.* To assess the biochemical integrity of electrotransfected muscles, we examined the activities of selected enzymes [phosphofructokinase, citrate synthase, 3β-hydroxyacyl-CoA dehydrogenase (3-HAD)] (17), muscle metabolites (glycogen, lactate), and creatine, creatine phosphate, and ATP, which were determined based on methods described by Hintz et al. (18).

STATISTICS. The data were analyzed using analyses of variance (i.e., independent groups and repeated measures ANOVA depending on the particular comparison). A least squares difference post hoc test was used to identify differences between specific conditions. All data are expressed as means ± SE.

## RESULTS

Surgery and electrotransfection had minor effects on the animals' body weights, with only a 2–4% weight loss during the first 2 days, followed by normal weight gain thereafter. These changes are less marked than in other experiments involving surgical intervention of skeletal muscle (34–36). The metabolic profile of electrotransfected muscle showed a small significant decrease in ATP (−20%, $P < 0.05$), creatine phosphate (−28%, $P < 0.05$), and total creatine (−14%, $P < 0.05$), and an increase in glycogen (+20%, $P < 0.05$) (data not shown). Although a reduction in 3-HAD activity did occur (−36%, $P < 0.05$), no changes were observed in the activities of phosphofructokinase and citrate synthase ($P > 0.05$, data not shown). Collectively, these data indicate that electrotransfection was well tolerated by the animals and caused only modest changes in some, but not all, markers of muscle metabolism.

*FABPpm in electrotransfected soleus muscle.* Successful electrotransfection of soleus muscle with the pTracer-FABPpm was indicated by the accumulation of the GFP reporter as detected by Western blotting (Fig. 1*A*). Electrotransfected muscle also resulted in an accumulation of FABPpm mRNA (Fig. 1*B*). The omission of much of the 5′ and 3′ untranslated region of FABPpm cDNA in pTracer-FABPpm allowed us to distinguish between endogenous FABPpm 2.4-kb mRNA and the shorter 1.9-kb transcripts derived from the plasmid vector.

FABPpm protein overexpression was directly related to the quantity of DNA administered (Fig. 2). In subsequent experiments 200 μg/muscle of FABPpm cDNA was used. There was a rapid increase in FABPpm protein over the course of 1 wk (Fig. 3*A*). After 1 day, FABPpm protein was increased +92% ($P < 0.05$). Maximum overexpression occurred between 3 days (+160%, $P < 0.05$) and 7 days (+142%, $P < 0.05$) (Fig. 3*A*). Electrotransfection with pTracer alone resulted in a small upregulation of FABPpm (+18%, $P < 0.05$) after 1 day. This did not increase further thereafter ($P > 0.05$, Fig. 3*A*) and was only a modest increase compared with the eightfold greater increase observed when pTracer-FABPpm was administered (Fig. 3). Importantly, the specificity of the FABPpm overexpression was demonstrated by the fact that the expression of other transport proteins (MCT1, FAT/CD36) and Cav-3 was not altered (Fig. 3*B*). Transfection of soleus muscle with FABPpm increased the total content of FABPpm (+142%) and its plasmalemmal content (+173%) to a similar extent (Fig. 4*A*). The activity of mAspAT was also increased (+42%, $P < 0.05$, data not shown).

*Effects of FABPpm overexpression on palmitate transport and metabolism.* In FABPpm-electrotransfected soleus muscles, the rate of palmitate transport into giant vesicles was

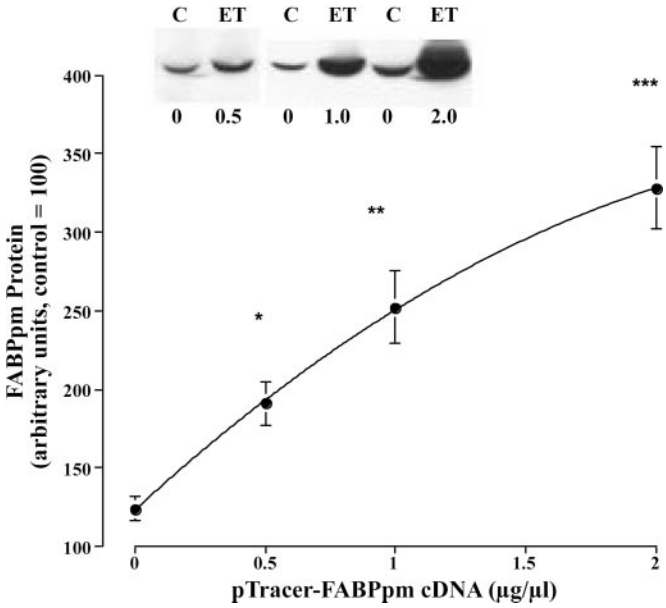


Fig. 2. Effect of different concentrations of pTracer-FABPpm DNA on FABPpm protein accumulation in soleus muscle after 7 days. Data are means ± SE ($n$ = 3–4 muscles at each point). *Inset*: a typical Western blot. *$P < 0.05$, FABPpm protein expression differs significantly from control (i.e., 100 μg > 0 μg). **$P < 0.05$, FABPpm protein expression differs significantly from preceding concentration (i.e., 200 μg > 100 μg). ***$P < 0.05$, FABPpm protein expression differs significantly from preceding concentration (i.e., 400 μg > 200 μg).

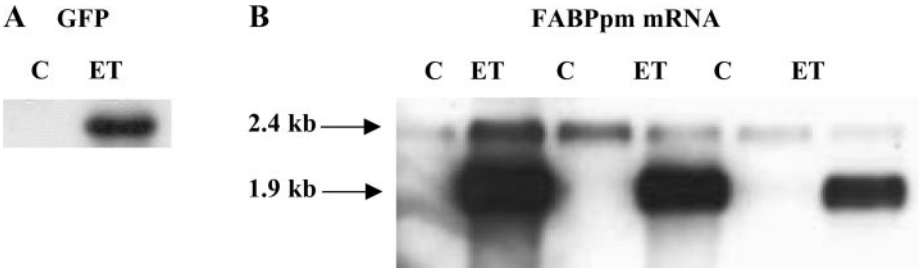


Fig. 1. Expression of green fluorescent protein (GFP) and plasma membrane-associated fatty acid binding protein (FABPpm) genes in electrotransfected rat soleus muscle. *A*: GFP protein accumulation in control (C) and electrotransfected (ET) muscle as determined by Western blotting. *B*: Northern blot demonstrating the accumulation of endogenous FABPpm mRNA (2.4 kb) in all soleus muscle samples (7 days after treatment) of three different animals, whereas pTracer-FABPpm-derived transcripts (1.9 kb) are found only in electrotransfected muscle.

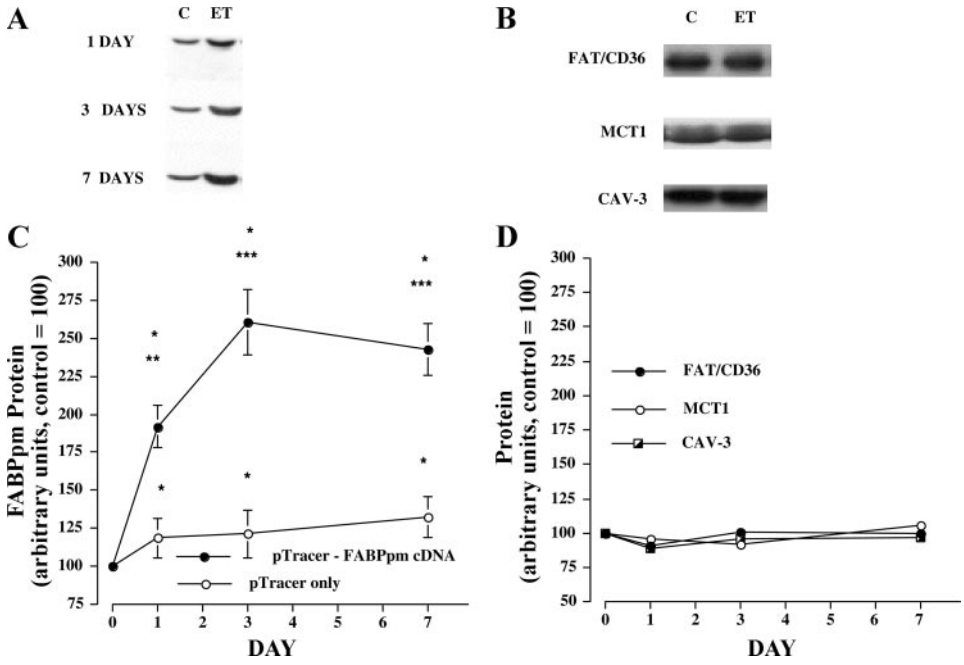


Fig. 3. Time course of FABPpm protein accumulation (*A* and *C*) in electrotransfected soleus muscle, as well as FAT/CD36, MCT1, and caveolin-3 (Cav-3) protein accumulation in the same muscles (*B* and *D*). *A*: representative Western blots of FABPpm at *days 1*, *3*, and *7*. *B*: representative Western blots of FAT/CD36, MCT1, and Cav-3 after 7 days (*B*). *C* and *D*: densitometric quantification of FABPpm (*C*) and other proteins (*D*). For clarity, error bars in *D* are not shown. Data are means ± SE; $n = 4$–6 muscles at each point. *$P < 0.05$, significantly different from *day 0*. **$P < 0.05$, significantly different from pTracer only on corresponding day. ***$P < 0.05$, significantly different from pTracer only on corresponding days, as well as from pTracer-FABPpm cDNA on *day 1*.

increased by 79% ($P < 0.05$, Fig. 4*B*). Since neither plasmalemmal FAT/CD36 ($P > 0.05$, Fig. 4*B*) nor H-FABPc (data not shown) were altered in FABPpm-electrotransfected muscle, the increase in the rate of palmitate transport in these muscles was not attributable to these proteins.

Along with the increase in palmitate transport, palmitate metabolism was also increased in FABPpm-transfected soleus muscles. The rate of palmitate incorporation into phospholipids was increased by 36% ($P < 0.05$, Fig. 5*A*). There was also a trend for an increase in the rate of palmitate oxidation (+20%, $P = 0.08$, Fig. 5*C*) in the muscles that overexpressed FABPpm. No change was observed in the rate of intramuscular palmitate incorporation into triacylglycerols (Fig. 5*B*). These changes in palmitate metabolism were more modest than the increase in plasmalemmal FABPpm and the rates of palmitate transport.

## DISCUSSION

These studies have shown that the expression of the fatty acid transporter FABPpm can be rapidly increased (1–7 days), in mature skeletal muscle, in vivo. Because FABPpm was also targeted to the plasma membrane, we were able to determine the functional consequences of overexpressing this protein on palmitate transport and metabolism in skeletal muscle, a metabolically important tissue. Importantly, these are the first studies to demonstrate that the overexpression of only FABPpm, in vivo, is sufficient to increase the rates of palmitate transport into giant sarcolemmal vesicles. Moreover, selected aspects of palmitate metabolism were also increased in muscles that overexpressed FABPpm. Thus these studies establish a clear role for FABPpm in palmitate flux across the plasma

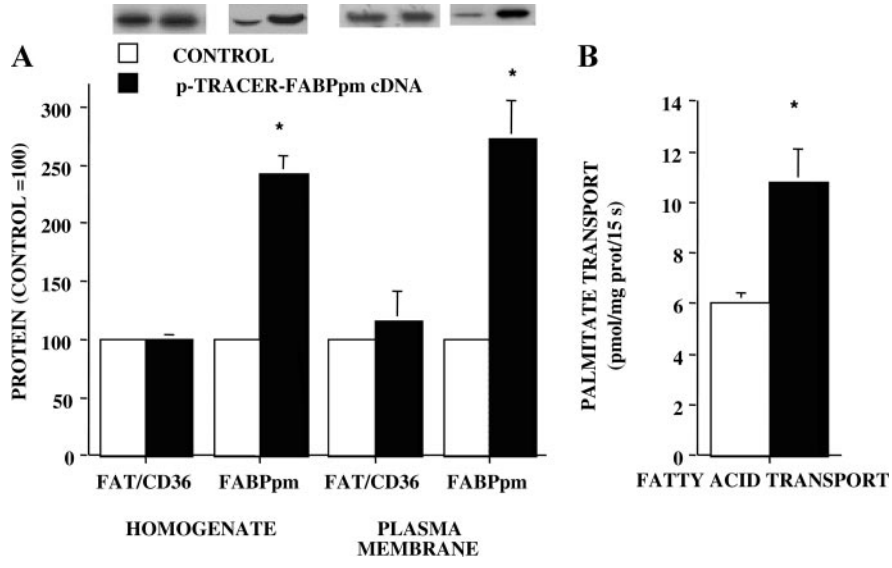


Fig. 4. Effects of electrotransfection of soleus muscle with FABPpm cDNA on homogenate and plasma membrane FABPpm and FAT/CD36 proteins (*A*) and palmitate transport into giant sarcolemmal vesicles (*B*). Data are means ± SE; $n = 3$–4 muscles at each point. For transport studies 3–4 soleii were pooled for each experiment, and fatty acid transport rates were determined in four such independent experiments in which muscles were pooled.

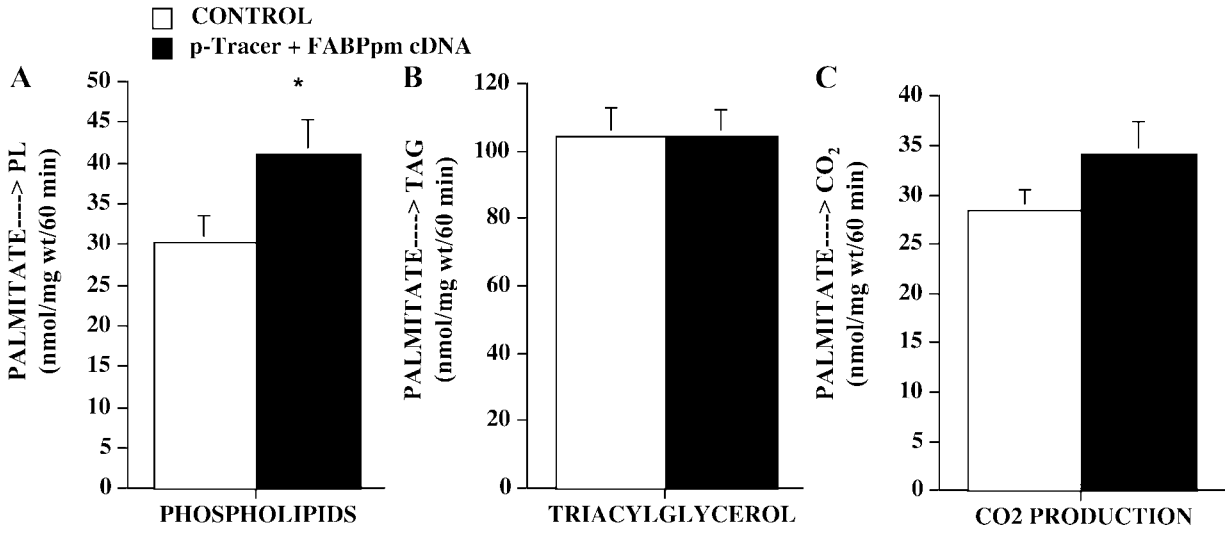


Fig. 5. Effects of electrotransfection of soleus muscle with FABPpm cDNA on the rates of palmitate incorporation into phospholipids (PL, *A*) and intramuscular triacylglycerols (TAG, *B*) and on the rate of palmitate oxidation (*C*) in isolated, intact soleus muscles. Data are means ± SE; $n = 4$ muscles for each treatment. *$P = 0.05$, pTracer-FABPpm cDNA vs. control. There was a trend for an increased rate of palmitate oxidation, pTracer-FABPpm cDNA vs. control ($P = 0.08$).

membrane in skeletal muscle, a process that also affects aspects of palmitate metabolism.

Electrotransfection of muscle has also been used by others to examine the function of specific genes (cf. Refs. 3, 26). The advantage of transfecting a single muscle with FABPpm is that *1*) whole body fuel homeostasis is not altered and *2*) the functional consequences of altered gene expression can be examined in isolated, mature skeletal muscles. In addition, since the contralateral muscle within the same animal serves as control, our approach also controlled for alterations in the substrate/endocrine milieu that might be occurring, as well as for possible alteration in food intake. The latter was not a problem as animals lost only minimal weight the first 2 days after surgery (<4%) and gained weight normally from *day 3* onward. Variation in FABPpm overexpression was low, with coefficients of variation (100 × SE/mean) ranging in all experiments from 6.6–11.7%. This is well within the range for many other biochemical measurements. We elected to perform the functional assessments of FABPpm overexpression by administering 200 μg FABPpm cDNA/muscle, which led to a +142% increase in FABPpm protein. This allowed us to control the overexpression of FABPpm and induce increments that were eightfold greater than the nonspecific 18% increase in control experiments and well beyond the experimental variability of 6.6–11.7%. Thus electrotransfection provided a highly effective means by which to overexpress FABPpm in a controlled manner, in vivo.

Although we used mAspAT cDNA to transfect muscle, it has been shown that 3T3 fibroblasts transfected with a cDNA for mitochondrial aspartate aminotransferase express FABPpm (22). It is known that FABPpm and mAspAT are the same protein (2, 43), which does not require alternative splicing of the mRNA (8). Immunogold labeling studies have shown that mAspAT/FABPpm is localized to the mitochondria and to the plasma membrane, as well as other sites in a number of tissues (9). FABPpm assists with fatty acid uptake across the plasma membrane (22), whereas mAspAT binds to the inner mitochondrial membrane, where this protein binds to the α-ketoglutarate dehydrogenase complex (15, 44) and catalyzes the following reversible reaction: glutamate + oxaloacetate ↔ aspartate + 2-oxoglutarate (25).

Specificity of FABPpm overexpression was demonstrated by the minimal, nonspecific changes induced in FABPpm, when only pTracer was administered, and by the lack of change in a number of other proteins, including the fatty acid transporter FAT/CD36. Moreover, appropriate subcellular targeting of FABPpm was confirmed by observing that plasmalemmal FABPpm was increased in transfected muscle. The increases in total FABPpm and plasmalemmal FABPpm were similar. Importantly, since neither the fatty acid transporter FAT/CD36 nor the fatty acid sink in giant vesicles, H-FABPc (7), were increased, the observed increase in palmitate transport rates cannot be attributed to concomitant changes in these proteins.

Although previous studies have associated changes in FABPpm with concomitant changes in rates of fatty acid transport across the plasma membrane (21, 27, 28, 39, 40, 41, 48), changes in other fatty acid transporters were not always examined or were also shown to be changed concomitantly (28, 27, 41). A more definitive role for FABPpm emerged from studies in which FABPpm antisera reduced palmitate uptake rates (31, 45). Isola et al. (22) have shown that transfection of 3T3 fibroblasts with mitochondrial aspartate aminotransferase cDNA increased fatty acid uptake rates, which they argued was due to the increased expression of FABPpm, not a secondary effect in 3T3 fibroblast metabolism. Our studies are the first to demonstrate that FABPpm overexpression in mammalian tissue increased the rates of palmitate transport across the plasma membrane. Unlike Isola et al. (22), we can eliminate entirely the concern that alterations in palmitate metabolism contributed to this increased flux, since we measured the rates of palmitate transport into giant sarcolemmal vesicles, which cannot metabolize fatty acids (5, 7, 31). Moreover, studies from our laboratory (5, 7, 31) have established that palmitate enters the lumen of the giant vesicle and is not merely bound to the plasma membrane. Indeed, protein-mediated fatty acid

uptake across the plasma membrane in skeletal muscle and heart is now a well-established process (5, 7, 31). But, the physical means by which FABPpm, and other proteins, bind and move fatty acids across the plasma membrane remains unknown.

A number of studies have also shown that fatty acid transport rates are regulated by other fatty acid transporters. In particular, FAT/CD36 is also a key fatty acid transporter in mammalian tissues such as heart, adipose tissue, and skeletal muscle. Expression of FAT/CD36 in Ob17Py fibroblasts increased fatty acid transport rates (21), and in transgenic mice, overexpression of FAT/CD36 in skeletal muscle increased palmitate oxidation during muscle contraction (20). A null mutation in FAT/CD36 reduced the uptake of fatty acids (10). In selected animal models (obese Zucker rats and streptozotocin-induced diabetes), in which plasmalemmal FAT/CD36, but not necessarily plasmalemmal FABPpm, was increased, there were concomitant changes in rates of fatty acid transport (27, 28). These observations began to suggest that while FABPpm may be a necessary component of the sarcolemmal fatty acid transport system, this protein might play only a facilitatory role to FAT/CD36. However, the present studies demonstrate clearly that an increase in plasmalemmal FABPpm alone, without a parallel increase in plasmalemmal FAT/CD36, can also result in an increased rate of fatty acid transport, although the rate of palmitate uptake was considerably less than the increase in plasmalemmal FABPpm.

The FABPpm-induced increase in palmitate transport also increased rates of fatty acid metabolism. Specifically, in transfected muscles, palmitate incorporation into phospholipids and palmitate oxidation were also increased. We (12) have previously shown that there is good relationship between palmitate uptake and metabolism, indicating that when more fatty acids enter the cell, the rates of fatty acid oxidation and esterification are increased. But, since, palmitate incorporation into triacylglycerols was not altered in the present study, the important implication is that an upregulation of FABPpm may not be associated with insulin resistance and type 2 diabetes, in which there is a gradual buildup of intracellular triacylglycerol depots (14, 35). Conversely, therapies designed to inhibit the transport function of FABPpm might not be effective in preventing triacylglycerol accumulation in type 2 diabetic muscles and, hence, may not have an antidiabetic potential.

In the present studies the increase in plasmalemmal FABPpm (+173%) exceeded the increase in palmitate transport (+79%) and in the rates of palmitate incorporation into phospholipids (+36%) and palmitate oxidation (20%). This suggests that the increase in FABPpm by itself is not optimally effective for increasing fatty acid transport and metabolism. We (31) have previously shown that fatty acid transport appears to involve the cooperation between FABPpm and FAT/CD36. In a rat heart muscle cell line (H9c2), fatty acid uptake was not increased when only FAT/CD36 was overexpressed, leading to the suggestion that a concomitant increase in FABPpm might also be necessary (46). The possible involvement of both FAT/CD36 and FABPpm in fatty acid oxidation is suggested by recent studies showing that FAT/CD36 is also found on the mitochondrial membrane (4), where mAspAT/FABPpm is also located (9). Thus conceivably FAT/CD36 and mAspAT/FABPpm might interact at this location to regulate fatty acid oxidation. Indeed, we have preliminary evidence for this (S. E. Campbell and A. Bonen, unpublished data). Thus the disparate increments observed in plasmalemmal FABPpm and in the rates of palmitate transport and oxidation may indicate that while an increase in plasmalemmal FABPpm can facilitate increased rates of fatty acid transport and metabolism, the FABPpm increase by itself is not sufficient to realize the full potential for increasing fatty acid transport and metabolism.

In summary, the present studies have shown that the fatty acid transporter FABPpm can be rapidly overexpressed in a single rat skeletal muscle. Overexpression of FABPpm increased the sarcolemmal content of FABPpm, resulting in increased rates of fatty acid transport across the sarcolemma and fatty acid metabolism. These studies provide the first evidence that FABPpm overexpression in mammalian tissue can increase fatty acid transport, which results in an increase in fatty acid metabolism.

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