Lipid-binding proteins and lipoprotein lipase activity in human skeletal muscle: influence of physical activity and gender

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Lipid-binding proteins and lipoprotein lipase activity in human skeletal muscle: influence of physical activity and gender. J Appl Physiol 97: 1209–1218, 2004. First published May 21, 2004; 10.1152/japplphysiol.01278.2003.—The protein and mRNA levels of several muscle lipid-binding proteins and the activity and mRNA level of muscle lipoprotein lipase (mLPL) were investigated in healthy, nonobese, nontrained (NT), moderately trained, and endurance-trained (ET) women and men. FAT/CD36 protein level was 49% higher (P < 0.05) in women than in men, irrespective of training status, whereas FAT/CD36 mRNA was only higher (P < 0.05) in women than in men in NT subjects (85%). Plasma membrane-bound fatty acid binding protein (FABP pm) content was higher in ET men compared with all other groups, whereas training status did not affect FABP pm content in women. FABP pm mRNA was higher (P < 0.05) in NT women than in ET women and NT men. mLPL activity was not different between gender, but mLPL mRNA was 160% higher (P < 0.001) in women than in men. mLPL activity was 48% higher (P < 0.05) in ET than in NT subjects, irrespective of gender, in accordance with 49% higher (P < 0.05) mLPL mRNA in ET than in NT subjects. A 90-min exercise bout induced an increase (P < 0.05) in FAT/CD36 mRNA (~25%) and FABP pm mRNA (~15%) levels in all groups. The present study demonstrated that, in the NT state, women had higher muscle mRNA levels of several proteins related to muscle lipid metabolism compared with men. In the ET state, only the gender difference in mLPL mRNA persisted. FAT/CD36 protein in muscle was higher in women than in men, irrespective of training status. These findings may help explain gender differences in lipid metabolism and, furthermore, suggest that the balance between gene transcription, translation, and possibly breakdown of several proteins in muscle lipid metabolism depend on gender.

fatty acid binding protein; fatty acid translocase/CD36; lipoprotein lipase; fatty acid transport protein 1; gene expression

CELLULAR UPTAKE OF LONG-CHAIN FATTY ACIDS (LCFA) occurs as a passive process dependent on the rate of cellular metabolism (63) and by a transport mechanism mediated by membrane-bound lipid-binding proteins (1, 8, 19). Among these proteins are particularly 1) plasma membrane fatty acid binding protein (FABP pm), a ~43-kDa protein located peripherally on the plasma membrane, which demonstrates an amino acid sequence partially identical to the mitochondrial enzyme aspartate aminotransferase (56, 57); 2) fatty acid translocase (FAT/CD36), an 88-kDa integral membrane glycoprotein with two predicted transmembrane domains, which is identical to glycoprotein IV or CD36 of human blood platelets and leukocytes (2, 21); and 3) fatty acid transport protein (FATP), a 63-kDa integral protein with six predicted transmembrane domains (11, 24, 50). There are also two proteins responsible for the intracellular transport of LCFA and long-chain acyl-CoA esters in the aqueous cytoplasm: the 14- to 15-kDa cytosolic fatty acid binding protein (FABP c) (19) and the 10-kDa acyl-CoA binding protein (ACBP) (15).

Within recent years, the interest in the regulation and function of the putative lipid-binding proteins has grown. In muscle cells, they may play a significant role in uptake and utilization of LCFA. However, the precise role of these proteins in mediating LCFA uptake and transport is not known, and the mechanisms of LCFA uptake into and transport within various mammalian cells may not be similar. From animal studies, it is now known that LCFA uptake is upregulated in heart, skeletal muscle, and adipose tissue in obesity (35) and diabetes (34) as well as during muscle contractions (9). The increase in LCFA uptake has been associated with an increased expression of FAT/CD36 (7, 25) and/or the relocation of FAT/CD36 from an intracellular depot to the plasma membrane (9, 39), although overexpression of FABP pm was also found to increase sarcolemmal transport of LCFA (14). However, in humans, few studies have examined whether expression of proteins involved in LCFA trafficking is subject to regulation. In human obesity (52) and after exercise training (29) or consumption of a fat-rich diet (47) in male volunteers, the muscle protein expression of FABP pm is increased. In obese, female Type 2 diabetics, the increase in muscle FABP pm induced by a low-calorie diet correlated with the weight loss and increase in resting fat oxidation (5). Short-term exercise training increased FAT/CD36 protein levels in a mixed group of men and women (59). These few studies suggest that the potential for LCFA uptake and intracellular trafficking may be increased in chronic metabolic diseases during caloric restriction and dietary interventions as well as during exercise training. However, it is likely that adaptations of lipid-binding proteins to diet and metabolic diseases are caused by different mechanisms and serve different purposes than adaptations to exercise training.
It was recently shown that mRNA of proteins involved in substrate metabolism in human skeletal muscle can be upregulated by one bout of exercise (45). The finding suggested that the cumulative effects of successive exercise bouts was responsible for the increase seen with exercise training in the level of proteins involved in oxidative metabolism. It is well known that endurance training results in a high capacity for LCFA oxidation (27), and the contribution of the different lipid sources [i.e., plasma LCFA, circulating very-low-density lipoprotein triacylglycerol (VLDL-TG) and intramuscular triacylglycerol] to energy production during exercise was recently shown to be gender dependent (48). Therefore, comparison of women and men at different training levels may reveal different expression patterns of fatty acid binding proteins, consistent with their putative role in LCFA metabolism. Moreover, gender- and/or training status-dependent responses to the increased metabolic load of acute exercise may also exist.

Lipoprotein lipase, located at the endothelial lumen of the capillary wall, is an enzyme responsible for the hydrolysis of circulating VLDL-TG. It is known that muscle lipoprotein lipase (mLPL) activity (mPLA) is enhanced during ingestion of a fat-rich diet and after exercise training (28, 30) and that mLPL mRNA expression is affected by dietary manipulations (46). Whether mLPL is affected by exercise training in respect to gender is less clear. Moreover, it is not known whether regulation of mLPL is associated with changes in lipid-binding proteins.

Therefore, the aim of the present study was to evaluate whether physical activity and gender, which both influence lipid metabolism, may also influence the different cellular lipid-binding proteins and mLPL. In an effort to elucidate the mechanism(s) underlying possible cellular adaptations associated with exercise training, we also examined the levels of lipid-binding proteins and mLPL. In an effort to elucidate the mechanism(s) underlying possible cellular adaptations associated with exercise training, we also examined the levels of lipid-binding proteins and mLPL. In an effort to elucidate the mechanism(s) underlying possible cellular adaptations associated with exercise training, we also examined the levels of lipid-binding proteins and mLPL. In an effort to elucidate the mechanism(s) underlying possible cellular adaptations associated with exercise training, we also examined the levels of lipid-binding proteins and mLPL. In an effort to elucidate the mechanism(s) underlying possible cellular adaptations associated with exercise training, we also examined the levels of lipid-binding proteins and mLPL.

**MATERIALS AND METHODS**

**Subjects**

Twenty-four women and 22 men, all healthy and nonsmoking, of different aerobic capacity were recruited to participate in the study (Table 1). All women were eumenorrheic, with a menstrual cycle length between 28 and 35 days, and none of them were taking oral contraceptives. Preliminary tests and the main exercise experiment in women were carried out in the midfollicular phase of their menstrual cycle (determined as day 7 to day 11 from onset of menstruation).

All subjects were fully informed about the risks associated with the study, and all of them gave written, informed consent. The study was approved by the Copenhagen Ethics Committee and conformed with the code of ethics of the World Medical Association (Declaration of Helsinki II).

**Experimental Protocol**

**Prescreening.** Peak oxygen consumption ($\dot{V}_{O_2}$ peak) was determined by an incremental bicycle exercise test, and training history and weekly training were determined from self-reports (questionnaire and activity record). Body composition was calculated by means of a three-compartment model (17). Body density was determined by hydrostatic weighing (53) with a correction for residual lung volume measured by the oxygen-dilution method (40). Bone mineral density was measured by dual-energy X-ray absorptiometry (DPX-IQ version 4.6.6, Lunar, Madison, WI).

Subjects were allocated to three groups according to training status: NT, moderately trained (MT), and ET subjects. NT had not participated in any regular physical training for at least 2 yr, but some participated in leisure-time physical activity once a week, and others occasionally bicycled for local transportation. Their $\dot{V}_{O_2}$ peak was $<45$ ml·kg body mass $^{-1}$·min $^{-1}$. MT had participated in regular exercise training per week for at least 2 yr and had a $\dot{V}_{O_2}$ peak of $45$–$55$ ml·kg body mass $^{-1}$·min $^{-1}$. ET participated in endurance-type physical training (cross-country skiing, running, rowing, cycling, and combined swimming and running) for at least $5$–$7$ h/wk, had trained for and participated in competition for at least $2$ yr, and had a $\dot{V}_{O_2}$ peak of $>55$ ml·kg body mass $^{-1}$·min $^{-1}$. The male groups were matched to the corresponding female groups according to their $\dot{V}_{O_2}$ peak/kg lean body mass and training history (Table 1).

**Dietary control.** When enrolled in the study, all subjects recorded their food intake by weighing all food consumed to the accuracy of 1 g on 5 nonconsecutive days during a period of 14 days. Habitual daily energy intake and nutrient composition of the diet were calculated by means of a computer program (Dankost 2000, Danish Catering Center, Copenhagen, Denmark). On the basis of these individual food records, all subjects consumed a similar, controlled, isocaloric diet during 8 days preceding the exercise experiment. The diet consisted of 65 energy percent carbohydrate, 20 energy percent fat, and 15 energy percent protein (Table 2).

**Exercise experiment.** On the morning of the experimental test, the subjects arrived at the laboratory by car, bus, or train after an overnight fast. On the preceding 2 days, subjects had abstained from

<table>
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<th>Table 1. Subject characteristics</th>
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<tr>
<td><strong>Women</strong></td>
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<tr>
<td><strong>NT</strong> (n = 7)</td>
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<tr>
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<tr>
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<td><strong>Body fat, %</strong></td>
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<td><strong>ml/kg LBM$^{-1}$·min$^{-1}$</strong></td>
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<td><strong>Training frequency, bouts/wk</strong></td>
</tr>
<tr>
<td><strong>Training duration, h/wk</strong></td>
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<tr>
<td><strong>Maximal CS activity, mmol/kg dry wt$^{-1}$·min$^{-1}$</strong></td>
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Values are means ± SE. NA, not applicable; CS, citrate synthase; NT, nontrained; MT, moderately trained; ET, endurance trained. *P < 0.001, main effect of gender; **P < 0.05, *P < 0.01, and ^P < 0.001 vs. NT; ^P < 0.001 vs. NT and MT.
exercise. All subjects performed an exercise test on a Krogh bicycle ergometer at the same relative workload corresponding to 60% VO$_2$ peak for 90 min. Before and at termination of exercise, a muscle biopsy was obtained from the vastus lateralis muscle from the same incision but with the needle directed in different angles. During exercise, expired air was collected frequently in Douglas bags for determination of oxygen uptake.

Methods

Muscle samples were frozen within 15 s in liquid nitrogen and stored at −80°C for subsequent analysis.

Maximal citrate synthase activity and mLPLA. Maximal citrate synthase (CS) activity and mLPLA were determined on the muscle biopsy obtained immediately before the bicycle exercise bout. Maximal CS activity was determined on ~15 mg wet weight of muscle tissue, which was freeze-dried and dissected free of all visible adipose tissue, connective tissue, and blood under a microscope. The muscle fibers were pooled, and ~2 mg of the dry muscle tissue were suspended in 800 μl of 0.3 M KPO$_4$ (pH 7.7), 0.05% BSA, and homogenized. CS activity was then measured fluorometrically on the homogenates at 25°C, pH 8.0, with acetyl-CoA as the substrate according to Lowry and Passonneau (33). mLPLA was determined on ~20 mg wet weight of muscle tissue according to Kiens and Lithell (30).

Western blotting. Protein levels of FABP$_{pm}$, FAT/CD36, muscle FABP$_c$, and ACBP were evaluated by Western blotting on the muscle biopsy obtained immediately before the bicycle exercise bout. Total crude membrane preparations were made for the measurement of FABP$_{pm}$ and FAT/CD36, whereas FABP$_c$ and ACBP were measured in a cytosolic preparation, as previously described (43), with minor modification. In short, muscle samples were homogenized (model Omni 2000, Omni, Warrenton, VA) in buffer containing (in mM) 250 sucrose, 30 HEPES (pH 7.5), 2 EGTA, and 2 PMSF. The homogenates were centrifuged at 200,000 g for 90 min, whereafter the supernatants containing cytosolic proteins were harvested and the pellets containing membrane proteins were resuspended in PBS with 1% Triton X-100. To determine the linearity range of the primary antibodies, Western blotting with protein gradients of a few samples was performed, and the optimal sample protein concentrations were assessed. Then, all samples were diluted to the same appropriate protein concentration and mixed with electrophoresis sample buffer before being subjected to SDS-PAGE and immunoblotting. The primary antibodies have been described elsewhere (12, 32, 42, 49). All primary antibodies revealed a single band at the expected molecular weight when used for Western blotting on human skeletal muscle (Fig. 1). Due to lack of useful FATP antibodies for Western blotting, FATP was not studied at the protein level. The secondary antibodies were alkaline phosphatase coupled (Zymed Laboratories). Antibody complexes were visualized, detected, and quantified with a chemiluminescence substrate (Attophos, Amersham, Uppsala, Sweden), a STORM scanner (Amersham), and ImageQuant software (Amersham), respectively.

RNA purification. Total RNA was isolated essentially as described by Chomczynski and Sacchi (13). Ten to 20 mg of muscle tissue were homogenized in 1 ml of TRIReagent (Molecular Research Center, Cincinnati, OH) using a polytron (Ultra-Turrax T8, Ika Labortechnik, Staufen, Germany). One hundred microliters of 1-bromo-3-chloropropane were added, and the sample was mixed thoroughly. After 5 min, the samples were centrifuged at 12,000 g at 4°C for 15 min, and the aqueous phase was precipitated with one volume of isopropanol for 5 min. After centrifugation at 12,000 g at 4°C for 8 min, the pellet was washed once with 1 ml of 75% ethanol. The dry pellet was resuspended in 20 μl of RNase-free water and stored at −20°C for later use. Total RNA yield was 614 ng/mg tissue (SD 88) with no difference between gender and training groups.

Single-stranded DNA probes. The Pfu polymerase (Stratagene, La Jolla, CA) was used to amplify PCR products (329–524 bp) from human muscle cDNA using the primers listed in Table 3. The PCR products were cloned into the Smal site of pBlueScript II SK(+) (3), resulting in the plasmids listed in Table 3. Templates for probes were made by PCR on the plasmids with M13 standard primers having the SmaI site of pBlueScript II SK(+) (3), giving rise to the sense strand 5′ biotinylated. The biotinylated

![Fig. 1](https://www.jap.org)
Table 3. Plasmids and primers used for Northern analysis

<table>
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<tr>
<th>Probe</th>
<th>ID</th>
<th>Orient</th>
<th>Sense Primer</th>
<th>Antisense Primer</th>
<th>GenBank</th>
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<td>GAAGCTATGCCTGCTCTAG</td>
<td>GCTGACATUGCCACTGCGAA</td>
<td>NM_002046</td>
</tr>
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ID, plasmid identification; orient, orientation in the vector (Z+ = same as lacZ, Z− = opposite lacZ); GenBank, GenBank accession number; FABP pm, plasma membrane fatty acid binding protein; FABP c, cytosolic fatty acid binding protein; FATP, fatty acid transport protein; mLPL, muscle lipoprotein lipase.

RESULTS

Effects of Gender and Training Status

Effects of gender and training status on mRNA levels were investigated only in NT and ET subjects.

Maximal CS activity. CS activity was not significantly different between female and male subjects, irrespective of training status (Table 1). In both women and men, a higher (P < 0.001) enzyme activity was found in ET compared with both NT and MT (Table 1).

FAT/CD36 mRNA and protein. Three different-sized bands for FAT/CD36 mRNA were seen in the Northern blot (Fig. 3A). A high degree of variability in the relative level of the two smaller isoforms was seen. However, no apparent link to gender or training status was observed, and the pattern was duplicated in the two biopsies from each subject. The two bands were too close together to allow quantitative separation, and the level of the third (larger) isoform covaried with the sum of the two smaller isoforms. Therefore, during quantification, all three bands were combined. FAT/CD36 mRNA was 85% higher in NT women than in NT men (P < 0.05; Fig. 3A), whereas no significant gender difference was observed in ET. The protein level of FAT/CD36 was higher (P < 0.05) in women than in men, irrespective of training status (NT: 76%; MT: 39%; ET: 32%) (Fig. 4A). There were no significant effects of training status on the mRNA and protein levels of FAT/CD36 (Figs. 3A and 4A).

Statistics

Data are presented as means ± SE unless otherwise stated. For variables independent of time, a two-way ANOVA was performed to test for effects of gender and training status. For variables measured before and after exercise, a three-way ANOVA, with repeated measures for the time factor, was performed to test for effects of gender and training status or changes due to time. When a significant main effect of training status was found, significant pairwise differences were performed using Tukey’s post hoc test. In all cases, a probability of 0.05 was used as the level of significance.

Fig. 2. GAPDH mRNA level in the vastus lateralis muscle expressed relative to 28S rRNA. NT, nontrained; ET, endurance trained; arb. units, arbitrary units. †Significantly different from NT of similar gender (P < 0.01).
**FABP<sub>pm</sub> mRNA and protein.** The FABP<sub>pm</sub> protein level was not significantly different between the three groups of women, although the FABP<sub>pm</sub> mRNA level was 38% lower ($P < 0.05$) in ET than in NT women (Figs. 3B and 4B). In men, the FABP<sub>pm</sub> protein level was higher in ET than in NT (45%) ($P < 0.01$) and MT (29%) ($P < 0.05$), which was not due to any significant differences at the mRNA level. FABP<sub>pm</sub> mRNA was 44% higher in NT women compared with NT men ($P < 0.05$), but this gender difference did not translate into significant gender differences on the protein level in NT subjects. The FABP<sub>pm</sub> protein level was 39% higher in ET men than in ET women ($P < 0.05$) without any significant difference in FABP<sub>pm</sub> mRNA.

**FABP<sub>c</sub> mRNA and protein.** In the NT state, but not in the ET state, the FABP<sub>c</sub> mRNA level was 101% higher in women than in men (Fig. 3C). No significant effects of training status were observed on FABP<sub>c</sub> mRNA. There were no significant effects of gender or training status on the FABP<sub>c</sub> protein level (Fig. 4C).

**FATP1 mRNA.** FATP1 mRNA was not significantly influenced by training status or gender (Fig. 3D).

**ACBP.** Significant effects of gender or training status on the ACBP level were not observed (Fig. 4D).

**mLPL mRNA and mLPLA.** mLPL mRNA was 49% higher ($P < 0.05$) in ET than in NT subjects irrespective of gender (Fig. 5A), and also mLPLA was 48% higher ($P < 0.05$) in MT and ET compared with the NT subjects, irrespective of gender (Fig. 5B). Moreover, mLPL mRNA expression was 160% higher in women than in men, irrespective of training status ($P < 0.001$), but this gender difference was not reflected in the mLPLA, which did not differ significantly between women and men in either group studied.

**Effects of Acute Exercise on mRNA Levels**

The effects of acute exercise on mRNA levels were evaluated only in NT and ET.

**FAT/CD36.** An exercise-induced increase in FAT/CD36 mRNA was observed in all groups (NT women: 21%; ET women: 2%; NT men: 41%; ET men: 31%; $P < 0.05$; Fig. 3A).
An exercise-induced increase in FABP<sub>pm</sub> mRNA was observed in all groups (NT women: 25%; ET women: 9%; NT men: 14%; ET men: 11%; P < 0.05; Fig. 3).

FABP<sub>c</sub> and FATP1. FABP<sub>c</sub> and FATP1 mRNA did not change significantly during exercise (Fig. 3, C and D).

mLPL. mLPL mRNA expression did not change significantly during exercise (Fig. 5A).

**DISCUSSION**

The present human study is the first in which 1) the protein and mRNA levels and 2) the exercise responsiveness of selected cellular lipid-binding proteins have been compared in skeletal muscle of women and men at different levels of physical activity. The results demonstrate that FAT/CD36 protein content was higher in muscle from women than from men, irrespective of training status. Basal mLPLA was not different between genders and was higher in trained than in NT individuals. Interestingly, the present findings revealed that mRNA levels in the vastus lateralis muscle of FAT/CD36, FABP<sub>pm</sub>, FABP<sub>c</sub>, and mLPL were significantly higher (85, 44, 101, and 160%, respectively) in NT female than in NT male volunteers. These differences were no longer apparent in the ET men and women, except in LPL mRNA expression, where the higher expression in ET women persisted.

**Higher Muscle FAT/CD36 Protein Expression in Women Than in Men**

With regard to the role of FAT/CD36, work within the last 25 years has established this protein as a multiligand receptor involved in several cellular functions, among which are fatty acid and lipid transport, utilization, and storage. Thus several studies have shown a positive relationship between the level of FAT/CD36 and the transport and/or metabolic capacity for LCFAs in rat skeletal muscle (10, 16, 25), giant sarcolemmal vesicle preparations from rat heart and skeletal muscle (36), incubated cardiac myocytes (37), and contracting cardiac myocytes in suspension (38), indicating that FAT/CD36 is involved in facilitating cellular LCFAs uptake in skeletal muscle. Recent data from both CD36 null mice (16) and muscle creatine kinase-CD36 transgenic mice, which overexpress FAT/CD36 in muscle (25), further support an important role of FAT/CD36 in lipoprotein and lipid homeostasis. It is therefore interesting that a gender difference in this lipid-binding protein was obtained in the present study. An explanation for the higher FAT/CD36 protein content in women than in men might be related to the fiber-type composition of skeletal muscle. Thus our laboratory has recently shown (62) by confocal microscopy that, in human skeletal muscle, FAT/CD36 is more abundantly expressed in type I than in type II fibers. Moreover, previous studies in rat skeletal muscle revealed a higher content of FAT/CD36 in red muscle than in white muscle when using light microscopy (64) or Western blotting on giant sarcolemmal vesicle preparations where FAT/CD36 expression was 39% higher in vesicles from red than from white muscle (10). Because the percentage of type I fibers in relation to fiber area was 22% higher in women than in men in the present study, which was reported by us in a previous communication (55), it seems likely that the 32–76% higher content of FAT/CD36 in...
skeletal muscle from women can, to some extent, be explained by the gender difference in muscle fiber-type composition. Furthermore, although the present data were obtained in the midfollicular phase of the menstrual cycle, where only estrogen concentrations were slightly higher in women than in men as previously reported in the same subjects (55), the influence of female sex hormones may also partly explain the generally higher protein expression of FAT/CD36 in female subjects. We are, however, not aware of any studies showing direct effects of female sex hormones on FAT/CD36 expression in skeletal muscle. The functional consequence of the gender difference in skeletal muscle FAT/CD36 might be a generally higher transsarcolemmal transport of LCFA and storage in skeletal muscle in women than in men. Interestingly, women have larger stores of intramuscular triacylglycerol compared with men (55). It is possible that the higher intramuscular triacylglycerol levels in women than in men is related to the higher FAT/CD36 expression in women, but further studies are required to verify this hypothesis.

**Influence of Physical Activity on Lipid-Binding Proteins**

It is well known that regular physical activity results in several adaptations in skeletal muscle. Accordingly, in the present study, \( V_{O_2} \text{ peak} \) was significantly higher in ET women (+37%) and men (+30%), who exercise trained –10 h each week, than in the NT women and men, who did no regular exercise training (Table 1). Moreover, the activity of CS, a key enzyme of the tricarboxylic acid cycle, which is usually higher in the trained compared with sedentary state, was also higher in the ET women (52%) and men (54%) compared with the NT subjects (Table 1). Our laboratory has previously demonstrated that exercise training results in an increase in mLPLA (30). In the present study, higher levels of mLPLA were also demonstrated in ET subjects than in NT subjects in both genders (Fig. 5B). Despite the markedly higher oxidative capacity of the trained subjects, the protein level of FAT/CD36 was not upregulated in the trained state in either men or women. In contrast, it was recently reported that 8 days of endurance exercise training induced a slight increase in FAT/CD36 protein content in skeletal muscle of three men and four women (59). This increase in FAT/CD36 protein content by short-term exercise training might be ascribed to the effect of the last exercise bout rather than training, because the biopsy in the study by Tunstall et al. (59) was obtained 24 h after the last exercise bout. The present study showed that 90 min of bicycle exercise induced an immediate increase in FAT/CD36 mRNA in both women and men (Fig. 3A). Taken together, these findings could suggest that exercise induces a transient transcription and translation of FAT/CD36 because, when measured 2 days after the last exercise session in the present study, FAT/CD36 protein content was not higher in the trained compared with the NT groups. In a recent study, the relationship between FAT/CD36 and insulin sensitivity was determined in CD36-deficient (CD36 \(^{-/-}\)) mice (20). The data revealed that whole body insulin-mediated glucose uptake during a hyperinsulinemic clamp was increased in CD36 \(^{-/-}\) mice compared with wild-type control mice (20). The increased whole body insulin sensitivity was due to an increased insulin sensitivity in skeletal muscle, whereas the liver in these mice was insulin resistant (20). Based on these findings, it is tempting to speculate that increased FAT/CD36 content in skeletal muscle is not a desirable adaptation and that it might, therefore, not be found with exercise training, in accordance with the findings in the present study.

In the present study, we found no effect of training status on FABP \(_{pm}\) protein in women in contrast to the findings in male subjects, where a significantly higher content of FABP \(_{pm}\) protein was seen in the most physically active men. These findings support our previous studies in men in which 3 wk of single-leg endurance exercise training induced an increase of 49% in muscle FABP \(_{pm}\) protein level, whereas no changes were observed in the contralateral NT muscle (29). This apparent gender difference in the adaptive response of FABP \(_{pm}\) to exercise training might explain why 8 days of endurance training did not change the amount of FABP \(_{pm}\) in a group of three men and four women (59). Alternatively, the training period might have been too short (59). The reason for the gender difference in FABP \(_{pm}\) response to exercise training is not clear. A higher level of FABP \(_{pm}\) in skeletal muscle of trained men compared with trained women might suggest that, during exercise, the utilization of fatty acids originating from plasma is higher in trained men than in trained women. In support of this, our recent findings show that, even though total fat oxidation during exercise was similar between genders, trained women utilized significant amounts of myocellular
triacylglycerol, whereas trained men did not (55), indicating that the contribution from blood-borne LCFA, which could originate from plasma LCFA or VLDL-TG, to oxidative metabolism was higher in trained men than in trained women (48).

The physiological reason for the higher FABP\textsubscript{pm} protein content only in the most active men and the lack of influence of exercise training on FAT/CD36 remains to be elucidated. From the present findings, it is tempting to suggest that these two proteins are under different regulation. In contrast, FABP\textsubscript{c} has been shown to be coexpressed with FAT/CD36 in heart (61), mammary cells (54), and adipocytes (2, 4). Similar to the findings on FAT/CD36, we did not observe a higher protein level of FABP\textsubscript{c} in the trained compared with the NT state. The lack of effect of exercise training on FABP\textsubscript{c} protein in skeletal muscle has been observed previously in rats (60) and in men (B. Kiens, unpublished observation). As was the case for FABP\textsubscript{c}, the ACBP level did not depend on training status, suggesting that the amounts of these cytosolic proteins were abundant and sufficient in trafficking long-chain acyl CoA esters and LCFA within the cell under the present circumstances. Our data are supported by recent findings in rats showing that endurance training did not affect the protein level of ACBP in skeletal muscle (18).

Basal levels of mLPLA were not different between genders in the present study, which was also observed in a previous study (44). Higher mLPLA in the trained state has previously been demonstrated in healthy male subjects (30, 58), and the present study reveals that similar changes are induced in association with regular physical activity in female volunteers. A close correlation between mLPLA and capillary density has been shown (30), which could partly explain the increase in mLPLA in the trained state. On the other hand, mLPLA was increased in male subjects during recovery from a single bout of exhaustive whole body exercise (31). In regard to mLPL mRNA, the present study did not show any changes immediately after stop of exercise in women or men, but the exercise bout might have induced a delayed increase in mLPL mRNA expression in accordance with previous findings during recovery (45). Thus it is tempting to speculate that the metabolic demands, induced during and/or after exercise, lead to an activation of the LPL gene in skeletal muscle and that even small, transient increases in the transcription rate of mLPL generate a cumulative increase in mLPL mRNA and subsequently in the protein content after regular exercise training for several weeks or months. Accordingly, not only mLPLA but also mLPL mRNA expression was higher in the ET than in the NT subjects in the present study. The physiological role of the exercise training-induced increase in mLPLA might be a higher capability to utilize circulating VLDL-TG during exercise (23, 27) and/or during recovery from exercise (41).

In regard to the expression of mRNA, we interestingly observed higher levels of FAT/CD36, FABP\textsubscript{pm} and FABP\textsubscript{c} mRNA expression in NT women than in NT men, without similar gender differences in the protein levels. Also, higher levels of mLPL mRNA were observed in women than in men in both NT and ET groups. These findings suggest that women generally have a higher mRNA level than men and that these higher mRNA levels are not necessarily translated into increased protein levels. Likewise, data from our laboratory have also revealed a higher mRNA level (but not enzyme activity) of the mitochondrial enzymes CS and β-hydroxyacyl-CoA dehydrogenase in female volunteers than in matched men (C. Roepstorff, P. Scherling, and B. Kiens, unpublished observation). It could be speculated that the gender difference in mRNA levels of the lipid-binding proteins observed in the present study is related to sex hormonal status. However, although this may be a possibility, it is unlikely to be the only explanation because the female subjects were all studied in the midfollicular phase of the menstrual cycle, in which the level of the sex hormones is close to that of men (55). Moreover, the differences in mRNA levels were not seen in the ET groups.

In summary, the principal finding of the present study is that, in skeletal muscle, FAT/CD36 protein content is higher in women than in men and that physical activity does not influence FAT/CD36 protein content. Also, for several of the lipid-binding proteins and for mLPLA, women were found to have increased muscle levels of mRNA compared with men. Finally, the FABP\textsubscript{c} in muscle are not influenced by gender or physical activity level. The physiological relevance of these findings awaits further investigation, but because altered expression of FAT/CD36 has been associated with obesity and insulin resistance (6) and atherosclerosis (22), the findings in the present study may have implications for understanding pathophysiology of the above-mentioned diseases.

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