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Biochemical and Biophysical Research Communications 346 (2006) 125–130
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Fatty acid binding protein 4 in human skeletal muscle

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Received 30 April 2006
Available online 24 May 2006

Abstract

The mechanisms that regulate intramyocellular triglycerol (IMTG) storage and mobilization are largely unknown. However, during the last decades several intracellular fatty acid binding proteins (FABPs) have been identified. FABP3 is the dominating FABP in skeletal muscle. Expression of additional FABPs is suggested from findings in FABP3-null mutated mice. In the present study, our aims were to investigate if FABP4 is expressed within skeletal muscle fibers and if FABP3 and FABP4 are more abundant in skeletal muscle fibers in endurance-trained than in control subjects. We show that FABP4 protein is expressed within the skeletal muscle fibers and that FABP4 mRNA and protein are more abundant in the endurance trained subjects. Still, FABP4 is markedly less expressed than FABP3, which is the generally accepted dominating FABP in skeletal muscle tissue.

Keywords: FABP3; FABP4; Fatty acid binding protein; Fat metabolism; Exercise; Gene expression; Muscle fiber types; Immunohistochemistry

The increased capacity for oxidizing fat in endurance-trained subjects has been known for a long time and is reflected by, for instance, the elevated 3-hydroxyacyl CoA dehydrogenase activity in the beta-oxidation pathway in trained muscles [1,2]. Also, the accumulation of intramyocellular triglycerol (IMTG) in the resting state has been demonstrated to be enhanced in endurance-trained subjects [3], and there seems to be a greater turnover of IMTG in the trained state [4]. During the last decades, several intracellular fatty acid binding proteins (FABPs) have been identified. They have important functions in the transport of intracellular fatty acids by increasing their solubility and have, in various experimental settings, been shown to enhance the transport of fatty acids from the cell membrane to the site of oxidation, i.e. the mitochondria, and to the site of esterification into IMTG. Interestingly, FABP4 has also been shown to be able to translocate into the nuclei and thereby influence gene transcription [5–7].

The FABP3 protein (also called heart fatty acid binding protein, HFABP) has been suggested to be the major FABP in skeletal muscle [8]. However, additional factors should be involved, since an increase in muscle fat metabolism still occurred in response to a contraction stimulus in FABP3-null mutated mice [9]. Numerous mechanisms have been described to be additive factors in muscle contraction induced changes of fatty acid metabolism, such as translocation of the fatty acid transporter FAT/CD36 from an intracellular pool to the plasma membrane [10]. Other fatty acid transport proteins located to the plasma membrane, such as FABPpm and FATP1, have been described in skeletal muscle [11]. However, recently we also revealed that FABP4 has been expressed in human skeletal muscle tissue at the mRNA level and that the FABP4 mRNA expression was markedly increased after a period of endurance training [12]. FABP4 has been assumed to be adipose-tissue specific and has for this

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0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.05.083

reason been used as an adipocyte marker [13]. Our finding of a training-induced increase in FABP4 may therefore relate to changes in the number of adipocytes in the skeletal muscle tissue. However, FABP4 may also be expressed within the muscle fiber itself, although it has not yet been investigated. In the present study, our aims were to investigate if FABP4 is expressed within skeletal muscle fibers and also whether training-induced changes at the mRNA levels are concurrent with changes at the protein level. Furthermore, we also wanted to study whether a concurrent increase of FABP3 and FABP4 exists in skeletal muscle tissue of highly endurance-trained individuals. In the present study, we show that FABP4 is expressed within the skeletal muscle fibers and FABP4 mRNA and proteins are more highly expressed in the elite endurance-trained athletes compared to control individuals. FABP4 is, however, approximately ten times less expressed at the mRNA level than to FABP3, the generally accepted dominating FABP in skeletal muscle tissue.

Methods

Experimental models. Six endurance-trained men (elite cyclists and triathletes) and six moderately active men (controls) were studied. Their mean (range) age, height, and weight were for the endurance trained group 23 (18–27) years, 183 (174–195) cm, and 74 (65–89) kg, and for the control group 24 (21–27) years, 181 (170–190) cm, and 73 (65–80) kg, respectively. The level of physical activity was assessed by their leisure-time physical activity, which totalled 23 (21–27) h of endurance training per week for the endurance-trained group and 2 (0–4) h of moderate intensity physical activity per week for the control group. Subjects' peak oxygen uptake (VO_2 peak) was determined on a cycle ergometer, in which the workload was increased stepwise until exhaustion and respiratory gases were continuously analyzed (Sensor Medics Vmax 229, Intra Medic AB, Bålsta, Sweden). VO_2 peak was 71 (64–79) $ml \times min^{-1} \times kg^{-1}$ for the endurance-trained group and 45 (40–50) $ml \times min^{-1} \times kg^{-1}$ for the control group. All procedures were explained in both oral and written forms to all subjects and were approved by the Committee of Ethics at the Karolinska Institutet, Stockholm, Sweden.

Skeletal muscle tissues. Biopsy samples were obtained at rest from vastus lateralis muscle by the percutaneous needle technique [14], frozen in isopentane pre-cooled in liquid nitrogen, and stored at −80 °C until mRNA and protein extraction or cross-cutting for immunohistochemistry.

Adipose tissue. Biopsy samples from six non-fat, non-diabetic individuals were taken from abdominal fat tissue during laparoscopic gallbladder operation, frozen in liquid nitrogen, and stored at −80 °C until RNA and protein extraction (Kindly supplied by Ann Kjellin, Karolinska University Hospital, Karolinska Institutet).

RNA extraction and reverse transcription. Total RNA was prepared with the acid phenol method [15] and quantified spectrophotometrically by absorbance at 260 nm. Integrity of total RNA was determined by 1% agarose-gel electrophoresis. Two micrograms of total RNA was reverse transcribed by Superscript reverse transcriptase (Life Technologies, Stockholm, Sweden) using random hexamer primers (Roche Diagnostics GmbH, Mannheim, Germany) in a total volume of 20 μl.

mRNA quantification. Real-time PCR was used to measure mRNA (ABI-PRISMA 7700 Sequence Detector, Perkin-Elmer Applied Biosystems Inc., Foster City, CA, USA). 18S rRNA was used as an endogenous control to correct for potential variation in RNA loading or efficiency of the amplification. Primers and probes were supplied as a TaqMan® Reagents kit and 18S (4310893E (Tamra)) FABP4 (Hs00609791_m1), and FABP3 (Hs00269758_m1) were supplied from Applied Biosystems. All reactions were performed in 96-well MicroAmp Optical plates. Amplification mixes (25 μl) contained the sample 5 μl cDNA diluted 1:100 and TaqMan Universal PCR Mastermix. Thermal cycling conditions included 2 min at 50 °C and 10 min at 95 °C before the PCR cycles began. Thermal cycling consisted of 40 cycles at 95 °C for 15 s and 65 °C for 1 min. Control experiments revealed approximately equal efficiencies over different starting template concentrations for target genes and endogenous control. For each individual, all samples were simultaneously analyzed in one assay run. Measurements of the relative distribution of FABP4 and FABP3 were performed for each individual; a C_T value was obtained by subtracting 18S rRNA C_T values from respective target C_T values. The expression of each target was then evaluated by $2^{-\Delta\Delta C_T}$ [16].

Western blotting. Muscle tissue samples from the cross-sectional study and adipose-tissue samples were homogenized as previously described [17]. The protein content was determined by Bradford assay using bovine serum albumine as a standard. Skeletal muscle tissue extracts from three control and three endurance-trained individuals (40 μg), and adipose tissue extract (10 and 20 μg) for positive control were loaded on a 15% sodium dodecyl sulfate (SDS)–polyacrylamide gel. Western blotting was performed as previously described [17]. The membranes were pre-incubated 1 h at room temperature with 5% milk in PBS containing 0.1% Tween 20 (PBS-T). The membranes were then incubated for 1 h at room temperature with mouse-anti human FABP4 diluted 1:1000 in 5% milk in PBS-T. After 3×10 min wash in PBS-T, the membranes were incubated with HRP-linked horse anti-mouse antibody (New England Biolabs) diluted 1:12000 for 1 h at room temperature. Immunocomplexes on the membranes were detected by enhanced chemiluminescences (ECL) system, SuperSignal® West Femto Maximum Sensitivity Substrate (Pierce) according to the manufacturer's protocol. The intensity of the immunocomplexes on the membranes were measured with an intensiometer Quick Northern Light precision illuminator (Imaging Research, St. Catharines, Ontario, Canada).

Immunohistochemistry. Cross-cuts (7 μm) from muscle biopsies from the cross-sectional training study (two subjects from each group) were fixed in 2.7% formaldehyde for 30 min, washed with PBS, and permeabilized with 0.5% Triton X-100 in PBS for 5 min. The expression of FABP4 was detected with a polyclonal rabbit antibody 4 μg/ml in PBS containing 1% BSA (Cayman Chemical #10004944), followed by Rhodamine Red ™-X conjugated donkey anti-rabbit antibody (Jackson ImmunoResearch Laboratories Inc.) diluted 1:160 in PBS, 1% BSA, incubated for 30 min at 37 °C. FABP3 was detected with a monoclonal rat antibody (MAB 1678, R&D Systems, Inc., 5 μg/ml) in the same way as FABP4 and followed by goat anti-rat IgG-FITC diluted 1:200 (Sc-2011, Santa Cruz Biotechnology). The sarcolemma was stained with an antibody for caveolin-3 (A-C, Sc-5310, Santa Cruz Biotechnology) followed by Rhodamine Red™-X conjugated donkey anti-mouse antibody (Jackson ImmunoResearch Laboratories Inc.). Nuclear DNA was stained with DAPI. Control staining without primary antibody using only the secondary antibody did not result in any staining of sections. Oil red O (500 mg) dissolved in 100 ml 60% triethyl phosphate (Sigma) was diluted 3:2 in deionized water and filtered before use. Sections were fixed as above and immersed in the Oil red O solution for 30 min. All slides were mounted in Vectashield hard mounting medium (Vector Laboratories).

Statistics. The mRNA data was analyzed using logarithmic-transformed ratios (target:endogenous control). Students *t*-test was used to test differences between the endurance-trained and control groups. Differences were considered significant at $p < 0.05$. Data are presented as means and SEM unless otherwise stated.

Results

FABP3 and FABP4 are expressed in skeletal muscle

The mRNA expression of FABP3 and FABP4 in skeletal muscle tissue of control subjects (normal level of physical activity) were 0.4 U/18S rRNA and 0.05 U/18S rRNA, respectively (Fig. 1). In adipose tissue the mRNA expression of FABP3 and FABP4 was 0.01 U/18S rRNA

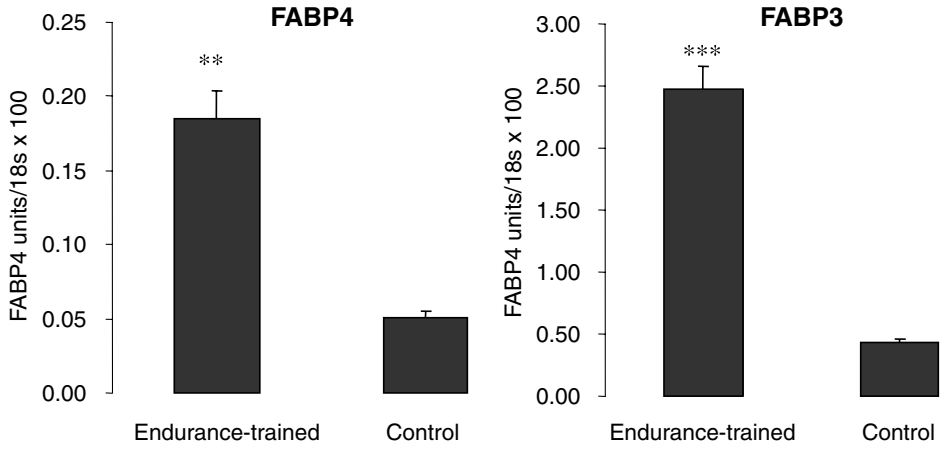

Fig. 1. Expression of FABP3 and FABP4 mRNA in skeletal muscle tissue in six endurance-trained and in six moderately active men (control). 18S rRNA was used as an endogenous control. ** and *** denote significant differences between endurance-trained and control subjects at the $p < 0.01$ and $p < 0.001$ levels, respectively. All data are expressed as means ± SEM.

and 13 U/18S rRNA, respectively. Thus, the mRNA expression ratio between FABP3 and FABP4 was about 10:1 in skeletal muscle. In adipose tissue the corresponding ratio was about 1:1000. To establish if the mRNA expression was associated with expression of protein, Western blots and immunohistochemistry were employed. Western blotting revealed FABP3 and FABP4 protein to be expressed in the skeletal muscle tissue (Fig. 2). The protein level of FABP4 in adipose tissue, used as a positive control for FABP4, was much higher than in the skeletal muscle tissue (Fig. 2). No FABP3 protein was detected in adipose tissue. With immunohistochemistry, FABP3 and FABP4 were located inside of the sarcolemma of the muscle fibers (Fig. 3). For FABP4, a subsarcolemmal location was confirmed which was further established by the caveolin-3 double staining (Fig. 4). FABP3 staining was associated with fibers that were more intensely stained for triglycerides by Oil red O (most likely type I fiber) (Fig. 3). This was also seen for FABP4, but visually not to the same extent. No adipocytes were identified in the muscle tissue by the Oil red O staining. Therefore, the expression of FABP4 in the current muscle tissue samples seemed not to be related to the presence of adipocytes.

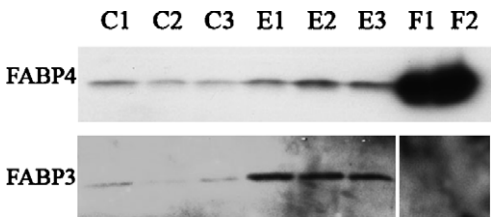

Fig. 2. Expression of FABP3 and FABP4 protein in skeletal muscle tissue in three endurance-trained (E) and in three moderately active men (C). The FABPs appeared near 14 kDa. Adipose-tissue protein extract is used as a positive control (F) for FABP4.

Higher expression of FABP3 and FABP4 in endurance-trained individuals

To investigate whether physical training influenced the FAPB4 expression in skeletal muscle, mRNA and protein expressions were measured in both endurance-trained and control individuals. The mRNA expressions of FABP4 and FABP3 were 3.8-fold ($p < 0.014$) and 5.6-fold ($p < 0.001$) higher in endurance trained compared to the control individuals, respectively (Fig. 4). The differences in mRNA were associated with an increase also at the protein level. The endurance-trained showed a two fold higher protein expression of FABP4 and an even higher expression of FABP3 compared to the control individuals (Fig. 2). In the endurance–trained individuals, a more intense FABP4 staining was seen with immunohistochemistry compared to controls, but with similar localization (Fig. 4).

Discussion

In the present study, we modify the general view of FABP4 as selectively expressed in adipocytes and we further report that an enhanced mRNA FABP4 level in endurance-trained athletes is concurrent with higher FABP4 protein levels. Still, FABP3 is much more abundant in skeletal muscle tissue compared to the expression of FABP4.

Recent observations indicate that the uptake and oxidation of free fatty acids as well as the turnover of the pool of intramyocellular triglycerids (IMTG) need to be carefully controlled and coordinated in human skeletal muscle. Failure in these mechanisms may result in pathologic metabolic processes such as attenuated glucose uptake [18,19]. We have recently reported that FABP4 mRNA expression in skeletal muscle tissue is increased with endurance training [12]. Although, based on its dominant expression in adipocytes, changes in FABP4 expression in skeletal muscle tissues, have mainly been attributed to changes in the

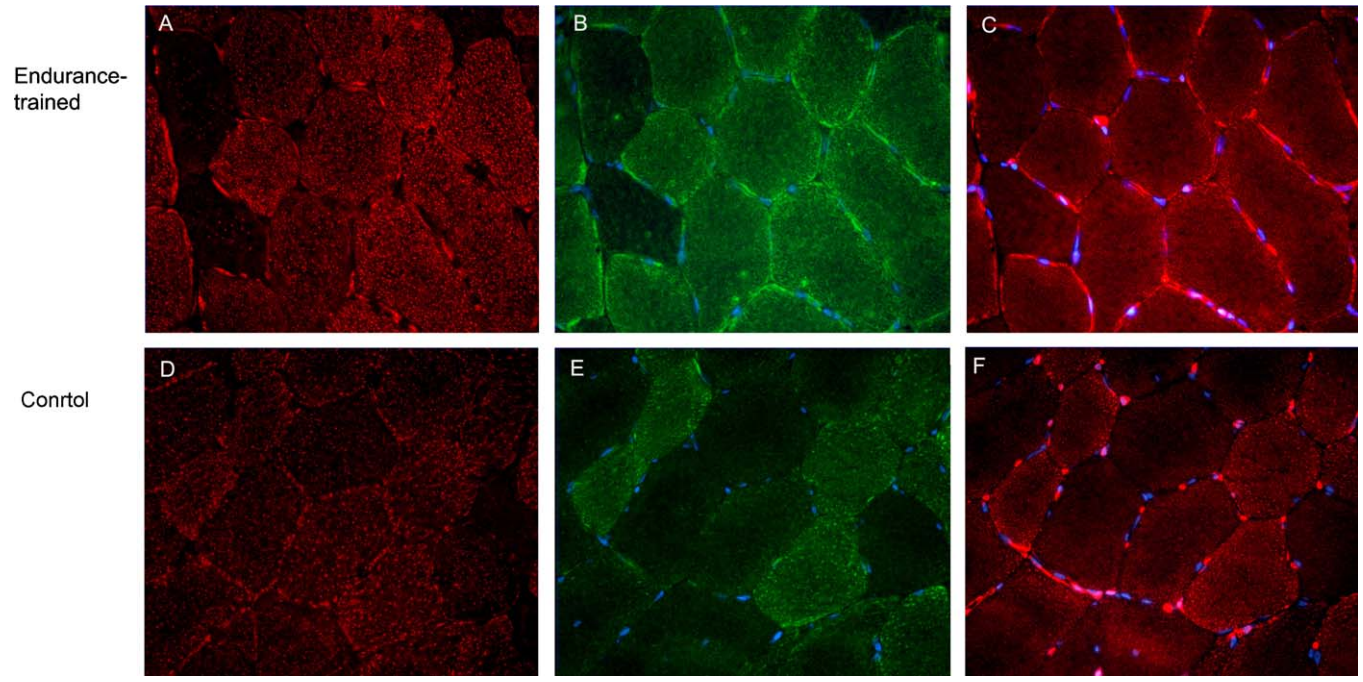

Fig. 3. Immunohistochemical staining of skeletal muscle tissue, cross-cut consecutive sections, in endurance-trained (A,B,C) and in moderately active (control) (D,E,F) individuals. In (A) and (D) triglycerides were stained with Oil red O, in (B) and (E) FABP3 was stained with the monoclonal antibody followed by FITC-labeled anti-rat secondary antibody, and in (C) and (F) FABP4 was stained with a polyclonal antibody followed by Rhodamine-labeled anti-rabbit secondary antibody. Nuclei were stained blue with DAPI.

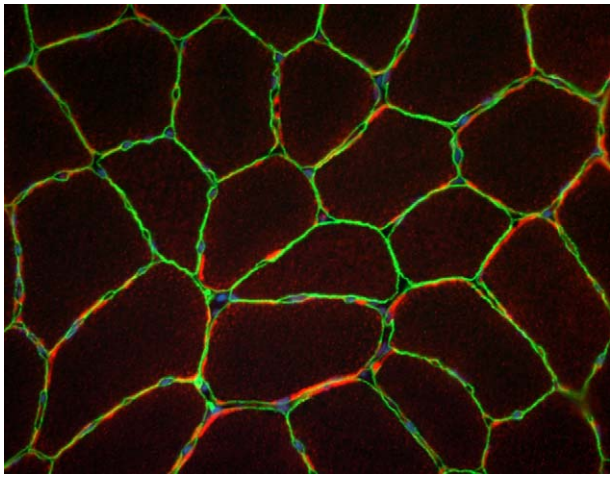

Fig. 4. Immunohistochemical staining of skeletal muscle tissue, cross-cut section, showing the FABP4 protein to be expressed inside the caveolin-3 protein expressing sarcolemma. Caveolin-3 was stained with a monoclonal antibody followed by FITC-labeled anti-mouse secondary antibody and FABP4 was stained with a polyclonal antibody followed by Rhodamine-labeled anti-rabbit secondary antibody. Nuclei were stained blue with DAPI.

number of adipocytes in the skeletal muscle tissue [13]. In fact, FABP4 expression has frequently been used as a cellular marker of the amount of adipocytes but the present observations challenge such statements. In the present study, concurrent with higher FABP4 mRNA levels, a higher protein expression was observed in the endurance-trained individuals and the protein was partly located to the subsarcolemma region of the fibers. This clearly implies that differences in the expression of FABP4 observed in various experimental settings not necessarily should be associated with a change in the amount of adipocytes.

FABP3 mRNA and protein levels were both higher in the endurance-trained individuals. This finding is supported by a recent report, in which an increase in FABP3 expression in human skeletal muscle tissue was demonstrated with endurance training [20]. It is tempting to speculate whether similar regulating factors would be involved in the regulation of FABP3 and FABP4. From analysis of the promoter sequences of each factor it seems, however, more likely that different signaling pathways are involved in the activation of the two proteins. For example, in the FABP3 promoter sequence, a Mef2 binding sequence has been identified [21], and in the FABP4 promoter sequence a PPARγ binding site has been recognized [22]. Both these transcription factors are functionally activated in exercised-skeletal muscle [23,24].

The mRNA levels of FABP4 in the present study was on the order of 100-fold higher in the adipose tissue than in skeletal muscle tissue and the ratio FABP3 to FABP4 was around 10-fold higher in the skeletal muscle tissue. These findings imply, as previously suggested, a predominant role of FABP3 as the fatty acid binding protein in skeletal muscle. Nevertheless, as earlier stated, fatty acid uptake in skeletal muscle was only reduced by 30% in FABP3 −/− mice, supporting an important role of other fatty acid binding

proteins in this process [9]. Thus, the present FABP4 mRNA and protein data together with our earlier report [12] support the possibility of an involvement of FAPB4. Still, due to the observed low abundance of FBAP4 compared to FABP3, it may be questioned whether FABP4 has any major physiological relevance in fatty acid handling or if other potential biological functions are of greater importance for FABP4 in skeletal muscle fibers.

The biologic roles for FABP4 are not yet fully known, but it has been reported that FABP4 functionally cooperates with PPARγ in adipocytes and in macrophages [25]. FABP4 enhances the transcriptional activity of the ligand of PPARγ through relocation of the ligand to the nucleus. Since PPARγ is expressed in the nuclei of human skeletal muscle fibers [26], a similar mechanism may be suggested for the biological effects of FABP4 in skeletal muscle fibers. Clarifications of the FABP4 contribution in fatty acid handling as well as in the other possible biologic actions in the skeletal muscle fibers are a challenge. Clearly, a better understanding of the regulatory pathways, including factors involved in fatty acid handling, is not only of basic scientific interest, but may also help direct us towards rational preventative measures and treatments.

In conclusion, we show that FABP4 protein is expressed within skeletal muscle fibers but to a lower extent than FABP3. FABP4 mRNA and protein expressions are much higher in endurance-trained compared to control individuals, indicating a physiological role of FABP4 such as in metabolic adaptation. The present observations also challenge the general notion of FABP4 as adipose-tissue specific.

Acknowledgments

We thank Ann Kjellin for supplying adipose-tissue biopsies. This work was supported by the Swedish Research Council, the Swedish National Centre for Research in Sports, and the Magnus Bergvall Foundation.

References

[1] E. Jansson, L. Kaijser, Substrate utilization and enzymes in skeletal muscle of extremely endurance-trained men, J. Appl. Physiol. 62 (1987) 999–1005.
[2] C. Roepstorff, N. Halberg, T. Hillig, A.K. Saha, N.B. Ruderman, J.F.P. Wojtaszewski, E.A. Richter, B. Kiens, Malonyl-CoA and carnitine in regulation of fat oxidation in human skeletal muscle during exercise, Am. J. Physiol. Endocrinol. Metab. 288 (2005) E133–E142.
[3] L.J.C. van Loon, R. Koopman, J.H.C.H. Stegen, A.J.M. Wagenmakers, H.A. Keizer, W.H.M. Saris, Intramyocellular lipids form an important substrate source during moderate intensity exercise in endurance-trained males in a fasted state, J. Physiol. (Lond) 553 (2003) 611–625.
[4] J. Rico-Sanz, M. Moosavi, E.L. Thomas, J. McCarthy, G.A. Coutts, N. Saeed, J.D. Bell, In vivo evaluation of the effects of continuous exercise on skeletal muscle triglycerides in trained humans, Lipids 35 (2000) 1313–1318.
[5] J. Storch, A.E. Thumser, The fatty acid transport function of fatty acid-binding proteins, Biochim. Biophys. Acta 1486 (2000) 28–44.
[6] A.W. Zimmerman, J.H. Veerkamp, New insights into the structure and function of fatty acid-binding proteins, Cell Mol. Life Sci. 59 (2002) 1096–1116.
[7] N.H. Haunerland, F. Spener, Fatty acid-binding proteins–insights from genetic manipulations, Prog. Lipid Res. 43 (2004) 328–349.
[8] J.F. Glatz, F.G. Schaap, B. Binas, A. Bonen, G.J. van der Vusse, J.J. Luiken, Cytoplasmic fatty acid-binding protein facilitates fatty acid utilization by skeletal muscle, Acta Physiol. Scand. 178 (2003) 367–371.
[9] B. Binas, X.X. Han, E. Erol, J.J. Luiken, J.F. Glatz, D.J. Dyck, R. Motazavi, P.J. Adihetty, D.A. Hood, A. Bonen, A null mutation in H-FABP only partially inhibits skeletal muscle fatty acid metabolism, Am. J. Physiol. Endocrinol. Metab. 285 (2003) E481–E489.
[10] A. Bonen, J.J.F.P. Luiken, Y. Arumugam, J.F.C. Glatz, N.N. Tandon, acute regulation of fatty acid uptake involves the cellular redistribution of fatty acid translocase, J. Biol. Chem. 275 (2000) 14501–14508, 10.1074/jbc.275.19.14501.
[11] J. Luiken, D. Miskovic, Y. Arumugam, J. Glatz, A. Bonen, Skeletal muscle fatty acid transport and transporters, Int. J. Sport Nutr. Exerc. Metab. (Suppl:S) (2001) 92–96.
[12] J.A. Timmons, O. Larsson, E. Jansson, H. Fischer, T. Gustafsson, P.L. Greenhaff, J. Ridden, J. Rachman, M. Peyrard-Janvid, C. Wahlestedt, C.J. Sundberg, Human muscle gene expression responses to endurance training provide a novel perspective on Duchenne muscular dystrophy, Faseb J. 19 (2005) 750–760.
[13] S.L. Coort, W.A. Coumans, A. Bonen, G.J. van der Vusse, J.F. Glatz, J.J. Luiken, Divergent effects of rosiglitazone on protein-mediated fatty acid uptake in adipose and in muscle tissues of Zucker rats, J. Lipid Res. 46 (2005) 1295–1302.
[14] J. Bergström, Muscle electrolytes in man, Scand. J. Clin. Lab Invest. 14 (1962) 1–110.
[15] P. Chomczynski, N. Sacchi, Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction, Anal. Biochem. 162 (1987) 156–159.
[16] J. Winer, C.K.S. Jung, I. Shackel, P.M. Williams, Development and validation of real-time quantitative reverse transcriptase-polymerase chain reaction for monitoring gene expression in cardiac myocytesin vitro, Anal. Biochem. 270 (1999) 41–49.
[17] H. Ameln, T. Gustafsson, C.J. Sundberg, K. Okamoto, E. Jansson, L. Poellinger, Y. Makino, Physiological activation of hypoxia inducible factor-1 in human skeletal muscle, Faseb J. 19 (2005) 1009–1011.
[18] C. Anderwald, E. Bernroider, M. Krssak, H. Stingl, A. Brehm, M.G. Bischof, P. Nowotny, M. Roden, W. Waldhausl, Effects of insulin treatment in type 2 diabetic patients on intracellular lipid content in liver and skeletal muscle, Diabetes 51 (2002) 3025–3032.
[19] D.A. Pan, S. Lillioja, A.D. Kriketos, M.R. Milner, L.A. Baur, C. Bogardus, A.B. Jenkins, L.H. Storlien, Skeletal muscle triglyceride levels are inversely related to insulin action, Diabetes 46 (1997) 983–988.
[20] B. Schmitt, M. Fluck, J. Decombaz, R. Kreis, C. Boesch, M. Wittwer, F. Graber, M. Vogt, H. Howald, H. Hoppeler, Transcriptional adaptations of lipid metabolism in tibialis anterior muscle of endurance-trained athletes, Physiol. Genomics 15 (2003) 148–157.
[21] Q. Qian, L. Kuo, Y.T. Yu, J.N. Rottman, A concise promoter region of the heart fatty acid-binding protein gene dictates tissue-appropriate expression, Circ Res 84 (1999) 276–289.
[22] T. Helledie, F. Surname, C. Jørgensen, M. Antonius, A.-M. Krogsdam, I. Kratchmarova, K. Kristiansen, S. Mandrup, Role of adipocyte lipid-binding protein (ALBP) and acyl-CoA binding protein (ACBP) in PPAR-mediated transactivation, Molecular and Cellular Biochemistry 239 (2002) 157–164.
[23] S.L. McGee, D. Sparling, A.-L. Olson, M. Hargreaves, Exercise increases MEF2- and GEF DNA-binding activities in human skeletal muscle, Faseb J. 20 (2006) 348–349.
[24] T.R. Koves, P. Li, J. An, T. Akimoto, D. Slentz, O. Ilkayeva, G.L. Dohm, Z. Yan, C.B. Newgard, D.M. Muoio, Peroxisome Proliferator-activated Receptor-{gamma} Co-activator 1{alpha}-mediated Metabolic Remodeling of Skeletal Myocytes Mimics Exercise

Training and Reverses Lipid-induced Mitochondrial Inefficiency 10.1074/jbc.M507621200, J. Biol. Chem. 280 (2005) 33588–33598.
[25] L. Makowski, G.S. Hotamisligil, Fatty acid binding proteins–the evolutionary crossroads of inflammatory and metabolic responses, J. Nutr. 134 (2004) 2464S–2468S.
[26] R.J. Tunstall, K.A. Mehan, G.D. Wadley, G.R. Collier, A. Bonen, M. Hargreaves, D. Cameron-Smith, Exercise training increases lipid metabolism gene expression in human skeletal muscle, Am. J. Physiol. Endocrinol. Metab. 283 (2002) E66–E72.