Skeletal muscle fatty acid transporter protein expression in type 2 diabetes patients compared with overweight, sedentary men and age-matched, endurance-trained cyclists.

Acta Physiol 2007, **190**, 209–219

Skeletal muscle fatty acid transporter protein expression in type 2 diabetes patients compared with overweight, sedentary men and age-matched, endurance-trained cyclists

M. M. A. L. Pelsers,[1] K. Tsintzas,[2] H. Boon,[3] K. Jewell,[2] L. Norton,[2] J. J. F. P. Luiken,[4] J. F. C. Glatz[4] and L. J. C. van Loon[1,3]

1 Department of Movement Sciences, Maastricht University, Maastricht, the Netherlands

2 Institute of Clinical Research, University of Nottingham Medical School, Nottingham, UK

3 Department of Human Biology, Maastricht University, Maastricht, the Netherlands

4 Department of Molecular Genetics, Maastricht University, Maastricht, the Netherlands

Received 3 October 2006,
revision requested 11 December 2006,
revision received 8 January 2006,
accepted 24 January 2007
Correspondence: M. Pelsers, PhD, Department of Movement Sciences, Maastricht University, PO Box 616, 6200 MD Maastricht, the Netherlands.
E-mail: maurice.pelsers@bw.unimaas.nl

Abstract

Aim: Membrane fatty acid transporters can modulate the balance between fatty acid uptake and subsequent storage and/or oxidation in muscle tissue. As such, skeletal muscle fatty acid transporter protein expression could play an important role in the etiology of insulin resistance and/or type 2 diabetes.

Methods: In the present study, fatty acid translocase (FAT/CD36), plasma membrane-bound fatty acid-binding protein (FABPpm) and fatty acid transport protein 1 (FATP1) mRNA and protein expression were assessed in muscle tissue obtained from 10 sedentary, overweight type 2 diabetes patients (60 $\pm$ 2 years), 10 sedentary, weight-matched normoglycemic controls (60 $\pm$ 2 years) and 10 age-matched, endurance trained cyclists (57 $\pm$ 1 years).

Results: Both FAT/CD36 and FATP1 mRNA and protein expression did not differ between groups. In contrast, FABPpm mRNA and protein expression were approx. 30–40% higher in the trained men compared with the diabetes patients ($P < 0.01$) and sedentary controls ($P < 0.05$).

Conclusions: Skeletal muscle FAT/CD36, FABPpm and FATP1 mRNA and protein expression are not up- or downregulated in a sedentary and/or insulin resistant state. In contrast, FABPpm expression is upregulated in the endurance trained state and likely instrumental to allow greater fatty acid oxidation rates.

Keywords CD36, exercise, FABPpm, FATP1, GLUT4, metabolism, muscle.

Numerous studies have reported strong associations between elevated plasma long-chain fatty acid (LCFA) concentrations, increased LCFA uptake, intramyocellular triacyl-glycerol (IMTG) accumulation and the development of insulin resistance and/or type 2 diabetes (Boden 1996, Roden *et al.* 1996, Pan *et al.* 1997, Perseghin *et al.* 1999, Kelley & Mandarino 2000, Shulmann 2000). However, the relationship between elevated IMTG stores and insulin resistance does not appear to be functional, as endurance trained athletes are markedly insulin-sensitive, despite elevated IMTG levels (Goodpaster *et al.* 1999, van Loon *et al.* 2004). The latter is explained by the fact that it is not the actual size or distribution of the IMTG pool but rather the imbalance between increased LCFA availability, uptake and/or decreased oxidation that is responsible for the development of skeletal muscle insulin resistance (Jensen 2002, Kelley

 doi: 10.1111/j.1748-1716.2007.01698.x

et al. 2002, van Loon *et al.* 2004, van Loon & Goodpaster 2006). In accordance, insights from various lipid infusion studies suggest that elevated LCFA uptake and/or impaired oxidation result in intramyocellular accumulation of LCFA metabolites (such as fatty acyl-CoA, ceramides and diacylglycerol), which are likely to induce defects in the insulin signalling cascade, causing skeletal muscle insulin resistance (Roden *et al.* 1996, Ellis *et al.* 2000, Kelley & Mandarino 2000, Itani *et al.* 2002, Yu *et al.* 2002, Adams *et al.* 2004).

Although LCFA uptake in skeletal muscle tissue can occur through passive diffusion (Hamilton *et al.* 2001), LCFA are also actively taken up by transporter proteins in the sarcolemma (Bonen *et al.* 1998a, Abumrad *et al.* 1999). Intramyocellular LCFA unloading of these transporters is facilitated by cytoplasmic plasma membrane-bound fatty acid-binding protein ($FABP_c$) and acyl-CoA binding protein (ACBP), which also serve as an intracellular LCFA buffer (Glatz *et al.* 2002). Therefore, LCFA transporter expression could represent an important factor modulating the balance between LCFA uptake and oxidation (Simoneau *et al.* 1999, Bruce *et al.* 2003, Bonen *et al.* 2004, Roepstorff *et al.* 2004, Koonen *et al.* 2005, Kiens 2006). Currently, three membrane-bound lipid-binding proteins have been identified that mediate skeletal muscle LCFA uptake (Kiens *et al.* 1997, Bonen *et al.* 1999, Roepstorff *et al.* 2004). Fatty acid translocase (FAT/CD36, 88 kDa), is a heavily glycosylated integral membrane protein with two predicted transmembrane domains and has been shown to be of major importance for the uptake of LCFA (Febbraio *et al.* 1999, Koonen *et al.* 2005). Plasma membrane-bound fatty acid-binding protein (FABPpm, 43 kDa) is located peripherally on the plasmamembrane and is identical to mitochondrial aspartate aminotransferase (Stremmel *et al.* 1985, Stump *et al.* 1993). The third one is fatty acid transport protein (FATP, 63 kDa), an integral protein with six predicted transmembrane domains (Schaffer & Lodisch 1994, Hirsch *et al.* 1998, Bonen *et al.* 1999, Pohl *et al.* 2004). So far six FATP isomers have been identified of which FATP1 is mainly expressed in skeletal muscle tissue (Hirsch *et al.* 1998).

The role of these transporter proteins in mediating LCFA uptake in muscle tissue is not yet fully understood, but various reports suggest that they are involved in the adaptive response to exercise (Kiens *et al.* 1997, 2004, Kiens 2006), as well as in the aetiology of insulin resistance and/or type 2 diabetes (Febbraio *et al.* 1999, Simoneau *et al.* 1999, Bonen *et al.* 2004, Kiens 2006). Previous studies have reported an upregulation of mixed muscle FABPpm protein content in obese subjects (Simoneau *et al.* 1999) and type 2 diabetes patients (Bruce *et al.* 2003). However, others have been unable to confirm these findings (Bonen *et al.* 2004). Furthermore, studies investigating the effects of training status on skeletal muscle LCFA transporter mRNA or protein expression have also reported contradictory findings (Tunstall *et al.* 2002, Kiens *et al.* 2004, Kiens 2006). Skeletal muscle sarcolemmal FAT/CD36 content is rapidly upregulated in response to acute exercise, whereas more long-term endurance training is needed to upregulate sarcolemmal FABPpm content (Tunstall *et al.* 2002, Kiens *et al.* 2004, Kiens 2006).

Because LCFA uptake is elevated in type 2 diabetes (Luiken *et al.* 2001, Bonen *et al.* 2004), we aimed to assess whether skeletal muscle mRNA and/or protein expression of the different LCFA transporter proteins are upregulated in type 2 diabetes patients. As it has recently been stressed that physically active subjects should act as controls when evaluating the effects of chronic metabolic disease on substrate use (Booth & Lees 2006), we assessed FAT/CD36, FABPm and FATP mRNA and protein expression in muscle tissue collected from both matched, sedentary controls as well as healthy, active men. As such, we provide a complete overview on skeletal muscle mRNA and protein expression of each of the LCFA transporter proteins in three well matched groups of subjects: 10 type 2 diabetes patients (60 ± 2 years), 10 matched sedentary, normoglycemic controls (60 ± 2 years), and 10 age-matched, endurance trained men (57 ± 1 years).

Methods

Subjects

A total of 10 sedentary, overweight type 2 diabetes patients (male, 60 ± 2 years), 10 sedentary, weight-matched, normoglycemic controls (male, 60 ± 2 years), and 10 age-matched endurance trained cyclists (male, 57 ± 1 years, minimum of 25 years cycling experience) were selected to participate in this study (Table 1). Type 2 diabetes patients and sedentary controls had not been engaged in any regular exercise programme. In contrast, the trained subjects exercised between three and four times a week, cycling at least 200 km $week^{-1}$. Type 2 diabetes patients and sedentary controls were matched for age, bodyweight and maximal oxygen uptake capacity. All type 2 diabetes patients were using oral blood glucose lowering medication (metformin with or without sulphonylurea derivatives). Type 2 diabetes status was verified with an oral glucose tolerance test (OGTT) according to WHO criteria (Alberti & Zimmet 1998). The study was approved by the local Medical Ethical Committee.

Journal compilation © 2007 Scandinavian Physiological Society, doi: 10.1111/j.1748-1716.2007.01698.x

Table 1 Subjects' characteristics

	Type 2 diabetes, $n = 10$	Sedentary controls, $n = 10$	Trained controls, $n = 10$
Age (years)	58.9 ± 2.5	60.0 ± 1.9	57.4 ± 0.8
Height (m)	1.79 ± 0.02	1.76 ± 0.01	1.75 ± 0.01
Body mass (kg)	93. ± 4.4	86.9 ± 1.9	77.7 ± 1.8†
BMI (kg m^{-2})	28.9 ± 1.2	27.5 ± 0.5	25.5 ± 0.7†
Body fat (%)	30.4 ± 1.8	28.9 ± 1.4	17.2 ± 11.2*†
Fat free mass (kg)	64.5 ± 2.4	61.7 ± 1.6	64.2 ± 11.2
Basal plasma LCFA (μM L^{-1})	626 ± 56	440 ± 34†	519 ± 56
Basal plasma glucose (mM)	8.95 ± 0.43	5.54 ± 0.16†	5.65 ± 0.08†
Plasma glucose $_{120min}$ (mM)	16.81 ± 1.0	5.34 ± 0.49†	5.28 ± 0.4†
Basal plasma insulin (mU L^{-1})	8.70 ± 1.01	7.86 ± 1.58	5.13 ± 0.56
Plasma insulin $_{120min}$	47.24 ± 9.61	48.40 ± 8.04	29.40 ± 6.34
HbA1c (%)	7.30 ± 0.3	5.83 ± 0.2†	5.78 ± 0.1†
VO_{2max} (L min^{-1})	2.9 ± 0.2	3.2 ± 0.2	3.8 ± 0.1*†
VO_{2max} (mL min^{-1} kg^{-1})	32 ± 2	37 ± 2	49 ± 2*†
VO_{2max} (mL min^{-1}/FFM)	45 ± 2	52 ± 3	59 ± 2*†
W_{max} (W)	205 ± 16	206 ± 18	300 ± 9*†
W_{max} (W kg^{-1})	2.20 ± 0.12	2.33 ± 0.17	3.87 ± 0.4*†
W_{max} (W/FFM)	3.10 ± 0.16	3.27 ± 0.20	4.67 ± 0.13*†
Maximal heartrate (bpm)	161 ± 4	164 ± 7	172 ± 3
Diagnosed with diabetes (years)	7 ± 1	–	–

Data are given as means ± SEM.
Body mass index (BMI) is calculated dividing body mass by height^2; Plasma glucose/insulin$_{120min}$ represents plasma glucose/insulin concentrations at $t = 120$ min.
*Significantly different from sedentary control group ($P < 0.05$); †significantly different from type 2 diabetes group ($P < 0.05$).

Screening

All subjects performed an OGTT. After an overnight fast, subjects arrived at the laboratory at 8 a.m. by car or public transportation. After 30 min of supine rest, a catheter (Baxter BV, Utrecht, the Netherlands) was inserted into an antecubital vein and a resting blood sample was drawn after which 75 g glucose (dissolved in 250 mL water) was ingested. Thereafter, blood samples were collected at $t = 120$ min without the use of a cuff. Plasma glucose concentrations (Table 1) were measured to assess glucose intolerance and/or type 2 diabetes according to the ADA guidelines of 2003 (Report of the Expert Committee 2003). Maximal power output (W_{max}) and maximal oxygen uptake capacity (VO_{2max}) were determined on an electronically braked cycle ergometer (Lode Excalibur, Groningen, the Netherlands) during an incremental exhaustive exercise test 2 weeks before muscle biopsy collection (Table 1). Oxygen uptake (VO_2) and carbondioxide production (VCO_2) were measured continuously (Oxycon®; Mijnhart, Breda, the Netherlands). Body composition was assessed using the hydrostatic weighing method in the morning after an overnight fast. Simultaneously, residual lung volume was measured by the helium-dilution technique using a spirometer (Volugraph 2000; Mijnhart, Bunnik, the Netherlands). Body weight was measured with a digital balance with an accuracy of 1 g (E1200; August Sauter GmbH, Albstadt, Germany). Body fat percentage was calculated using Siri's equation (Siri 1956). Fat free mass (FFM) was calculated by subtracting fat mass (FM) from total body weight (Table 1).

Diet and physical activity prior to muscle biopsy collection

All subjects were instructed to refrain from strenuous physical activity for 2 days before biopsy collection, and recorded dietary intake for 2 days. The evening before muscle biopsy collection, all subjects received the same standardized meal [4.2 kJ kg^{-1}; consisting of 61 Energy% (En%) carbohydrate, 24 En% fat and 15 En% protein]. There were no differences in daily energy intake between groups during the days before the tests (8.5 ± 0.6, 9.1 ± 2.0 and 9.3 ± 0.8 MJ day^{-1} in the type 2 diabetes patients, sedentary- and trained-control group, respectively). Macronutrient composition of the diet consisted of 54.9 En% carbohydrate, 28.8 En% fat and 16.3 En% protein in the type 2 diabetes patients, 52.8 En% carbohydrate, 31.9 En% fat and 15.3 En% protein in the sedentary controls and 52.4 En% carbohydrate, 29.2 En% fat and 18.4 En% protein in the trained controls. The protein intake was significantly higher in the trained controls (19 ± 1 vs. 16 ± 0

 doi: 10.1111/j.1748-1716.2007.01698.x

En%) compared with the diabetes patients and sedentary controls ($P < 0.05$).

Research protocol

Following an overnight fast, subjects arrived at the laboratory in the morning by car or public transportation. Height was determined and body mass was measured with a digital balance with an accuracy of 0.001 kg (E1200). Thereafter, a fasting blood sample was collected from an antecubital vein. After 30 min of supine rest, an anesthetic (1% xylocaine) was injected locally in skin, soft tissue below, and in the muscle fascia in the middle region of the *vastus lateralis* muscle. Thereafter, a small incision (5 mm) was made through the skin and the fascia at approx. 15 cm above the patella. An adapted Bergström needle was inserted to a depth of approx. 2–3 cm below the entry of the fascia and a muscle sample (appro. 60 mg) was obtained by suction. Muscle tissue was freed from any visible non-muscle material. Thereafter, one portion was immediately frozen in liquid nitrogen; another portion was frozen immediately in nitrogen-cooled isopentane and embedded in Tissue-Tek (Sakura, Zoeterwoude, the Netherlands). Both samples were stored at −80 °C until further analyses.

Protein extraction

For protein extraction and quantification, muscle samples (20–30 mg) were homogenized (1.5–10% w/v) in Hepes-buffer (50 mM Hepes, 1 mM EDTA, 10% glycerol, 10 mM NaF, 1 mM activated Na_3VO_4, 150 mM NaCL, 1% Triton X-100, pH 7.5) using an Ultra-Turrax homogenizer (2 × 15 s; IKA Werke, Breisgau, Germany). After 20 min incubation on ice and subsequent centrifugation for 20 min at 4 °C (10 000 ***g***, Eppendorf), supernatant was used for total protein quantification with the Pierce micro-BCA assay (23235; Pierce, Rockford, IL, USA) applying bovine serum albumin as standard.

Western blot analyses

For immunoblot analyses, either 10 μg (FAT/CD36 and FABPpm) or 50 μg (FATP1) protein of the membrane-extract was loaded on a 10% SDS-PAGE gel (Biorad, Hercules, CA, USA) and run for 50 min at 200 V. After blotting on nitrocellulose membrane for 90 min at 100 V, membranes were blocked for 1 h at room temperature with either bovine serum albumin (Sigma A4503, St. Louis, MO, USA) for FAT/CD36 or non-fat dry milk (NFDM) for FABPpm and FATP1. After washing with TBS-Tween, membranes were incubated o/n at 4 °C with primary antibodies for FAT/CD36 (MO25, 1/20 000 in TBS-tween, provided by N.N. Tandon) and FABPpm (1/3000 in NFDM, provided by J. Calles-Escandon), as previously described (Bonen *et al.* 1998b) and for FATP1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1/4000 in NFDM). After washing with TBS-Tween, secondary complexes were generated using anti-mouse IgG horseradisch-peroxidase conjugated antibody (1/20 000; Dako Cytomation, Glostrup, Denmark) for FAT/CD36 and anti-rabbit horseradisch-peroxidase conjugated antibody for FABPpm (1/3000; Chemicon, Temecula, CA, USA) and FATP1 (1/3000; Santa Cruz Biotechnology). Enhanced chemiluminescense detection (ECL) was performed with band densities obtained by densitometry. A control sample was run on each gel as loading control. ECL data of this sample was set as 100% and compared with all other samples.

Immunohistochemistry

For qualitative immunohistochemical staining, multiple serial sections (5 μm) from tissue-tek embedded samples were thaw mounted together on uncoated, pre-cleaned glass slides. To visualize FAT/CD36, FABPpm and FATP1, tissue slides were first stained overnight at 4 °C with primairy antibodies to FAT/CD36 and FATP1 (Santa Cruz Biotechnology) and to FABPpm. After washing with TBS/Tween, secondary antibodies labelled with either IgA-FITC (FAT/CD36) or IgG-FITC (FABPpm and FATP1) were added for 30 min incubation followed by three washing steps with TBS before analysis. CD31 antibody was added to allow membrane localization.

Real-time RT-PCR

For quantitative real-time PCR, total RNA was extracted from 10 to 20 mg of muscle tissue using Trizol reagent (Invitrogen, Breda, the Netherlands). Total RNA quantification was performed with the Ribo-green assay (Molecular Probes, Breda, the Netherlands). The first-strand cDNA was synthesized from 500 ng of total RNA using random primers (Promega, Madison, WI, USA) and Moloney Murine Leukemia Virus Reverse Transciptase (M-MLV RT) (Promega). Samples were subjected to quantitative real-time PCR using TaqMan probes (Sigma) and primer sets (synthesized by J. Keyte, University of Nottingham) for FAT/CD36, FABPpm and FATP1. The ABI PRISM 7000 system was used for the reaction and detection (Applied Biosystems). The probes were labelled with FAM at the 5′ end and with TAMRA at the 3′ end. PCR amplification was performed in a total volume of 25 μL containing 5 μL of diluted (one in four) cDNA sample, 300 nM of each primer, 200 nM of Taqman probe, and 12.5 μL of

Journal compilation © 2007 Scandinavian Physiological Society, doi: 10.1111/j.1748-1716.2007.01698.x

Table 2 Sequences for primers and Taqman probes used for real-time PCR

Gene	Forward primer	Probe	Reverse primer
FAT/CD36	CTGGAGTCTGGAATTCAGAACG	TCCTGCAGGTTCAGTGCCCCC	GAAGTGAGGATGGGAGAGAAACA
FABPpm	CACATCACCGACCAAATTGG	ATGTTCTGTTTCACAGGGCTAAAGC	AGCCGCTCCACCTGTTCA
FATP1	CGTCCTCCGCAAGAAATTCTC	CAGCCGCTTCTGGGACGACTGC	CTGAACCACCGTGCAGTTGT

TaqMan Universal PCR Master Mix. For each reaction, the polymerase was activated by pre-incubation at 95 °C for 10 min. Amplification was then performed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s. The FAT/CD36, FABPpm and FATP1 cDNA quantity in each sample was normalized to the housekeeping gene hydroxymethylbilane synthase (HMBS) mRNA. The probe and primers for HMBS were obtained from PE Applied Biosystems (Pre-developed TaqMan Assay Reagents Control kits) (Table 2). All other probes and primer sets (Table 2) were designed by K. Jewell (University of Nottingham). Real-time PCR was carried out in triplicate for each sample.

Statistics

All data are expressed as means ± SEM. To compare energy expenditure, food intake, fatty acid transporter mRNA and protein expression between groups, a one-factor analysis of variance (ANOVA) was applied. A Scheffé's *post hoc* test was applied in case of a significant F-ratio to locate specific differences. Significance was set at the 0.05 level of confidence.

Results

Subjects' characteristics

Fasting blood glucose and LCFA concentrations as well as HbA1c content were significantly higher in the type 2 diabetes patients when compared with the sedentary and/or trained control groups (Table 1). Type 2 diabetes patients and sedentary controls were matched for age, maximal power output (W_{max}), maximal oxygen uptake and body composition. The endurance trained group showed a significantly greater maximal power output (W_{max}) and maximal oxygen uptake capacity, expressed either per kg lean body mass (LBM) or per kg bodyweight (BW), when compared with the sedentary controls and type 2 diabetes patients ($P < 0.05$).

FAT/CD36

Total skeletal muscle FAT/CD36 protein content did not differ between groups (Fig. 1a). FAT/CD36 mRNA expression did not show any significant differences between type 2 diabetes patients and sedentary and/or endurance trained controls (Fig. 1b). Immunohistochemical staining showed FAT/CD36 expressed both in endothelial cells and the sarcolemma (Fig. 4a).

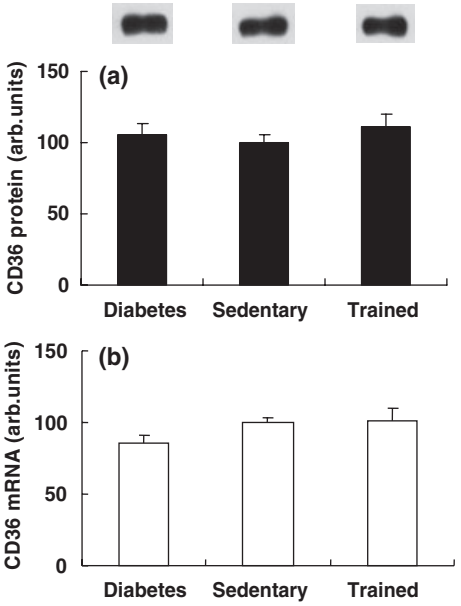

Figure 1 Mixed muscle total protein (a) and mRNA, (b) content of FAT/CD36 measured in type 2 diabetes patients, age- and BMI matched sedentary controls and endurance trained active controls. Representable Western blot is shown (a). Data for each gene were normalized to HMBS and expressed as mean ± SEM. No significantly differences between groups.

FABPpm

Total skeletal muscle FABPpm protein content was significantly higher (30%) in the endurance trained group when compared with the sedentary controls and type 2 diabetes patients ($P < 0.01$; Fig. 2a). In accordance, FABPpm mRNA expression was significantly higher (28–37%) in the endurance trained group compared with the sedentary controls and type 2 diabetes patients ($P < 0.05$; Fig. 2b). Immunohistochemical staining showed FABPpm expressed in endothelial cells and in the sarcolemma (Fig. 4b, green dots). Orange dots indicate CD31 staining on endothelial cells.

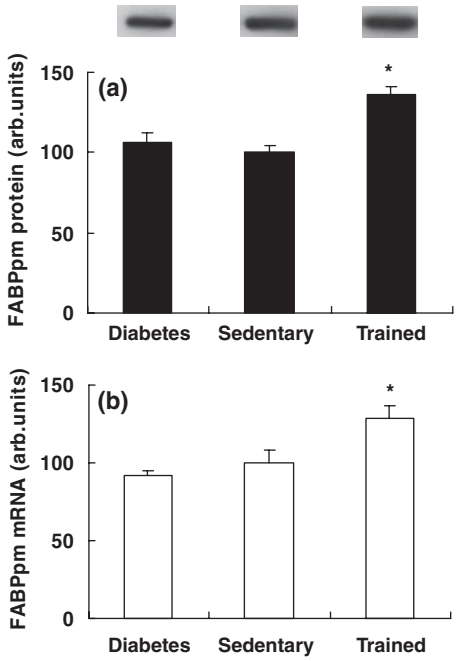

Figure 2 Mixed muscle total protein (a) and mRNA (b) content of FABPpm measured in type 2 diabetes patients, matched sedentary and endurance trained controls. Representable Western blot is shown (a). Data for each gene were normalized to hydroxymethylbilane synthase (HMBS) and expressed as mean ± SEM. *Significantly higher than in diabetes patients and sedentary controls $P < 0.01$.

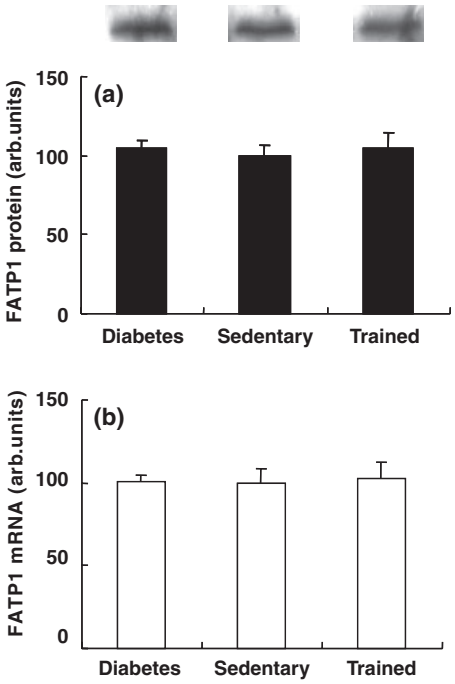

Figure 3 Mixed muscle total protein (a) and mRNA (b) content of FATP1 in type 2 diabetes patients, matched sedentary and endurance trained controls. Representable Western blot is shown (a). Data for each gene were normalized to hydroxymethylbilane synthase (HMBS) and expressed as mean ± SEM. No significant differences between groups.

FATP1

Total skeletal muscle FATP1 mRNA and protein content did not differ between groups (Fig. 3a, b). Immunohistochemical staining showed FATP1 located in the sarcolemma and endothelial cells (Fig. 4c).

No significant correlations between muscle transporter contents were observed, (FAT/CD36-FABPpm: $R^2 = 0.2433$), (FAT/CD36-FATP1: $R^2 = 0.1501$) and (FATP1-FABPpm: $R^2 = 0.0389$).

Discussion

In obesity and/or type 2 diabetes, a structural imbalance between LCFA availability, uptake, storage and/or oxidation is responsible for the development of skeletal muscle insulin resistance (Roden *et al.* 1996, Pan *et al.* 1997, Bonen *et al.* 1998a, Goodpaster *et al.* 1999, Perseghin *et al.* 1999, Kelley & Mandarino 2000, Shulmann 2000, Kelley *et al.* 2002, van Loon *et al.* 2004, van Loon & Goodpaster 2006). Elevated LCFA uptake and/or impaired LCFA oxidation lead to intramyocellular accumulation of FA metabolites (such as fatty acyl-CoA, ceramides and diacylglycerol), which can induce defects in the insulin signaling cascade, causing skeletal muscle insulin resistance (Roden *et al.* 1996, Ellis *et al.* 2000, Kelley & Mandarino 2000, Itani *et al.* 2002, Yu *et al.* 2002, Adams *et al.* 2004). Skeletal muscle LCFA transporters play an important role in modulating the balance between plasma LCFA availability and subsequent LCFA storage and/or oxidation by mediating LCFA uptake (Luiken *et al.* 2001, Bonen *et al.* 2002). As such, skeletal muscle LCFA transporter content could represent an important factor in the development and/or progression of skeletal muscle insulin resistance. The present study compares mRNA and protein expression of the LCFA transporter proteins in muscle tissue obtained from overweight, type 2 diabetes patients, matched sedentary controls, and age-matched, endurance-trained cyclists. No differences in muscle FAT/CD36, FABPpm and FATP1 mRNA and protein expression were observed between type 2 diabetes patients and matched, sedentary controls. As such, we show that FAT/CD36, FABPpm and FATP1 are not expressed differently in the type 2 diabetes state when subjects are matched for maximal oxygen uptake capacity. Bruce *et al.* (2003) reported comparable results for FAT/CD36 but showed a lower FABPpm content in matched elderly controls vs. diabetes

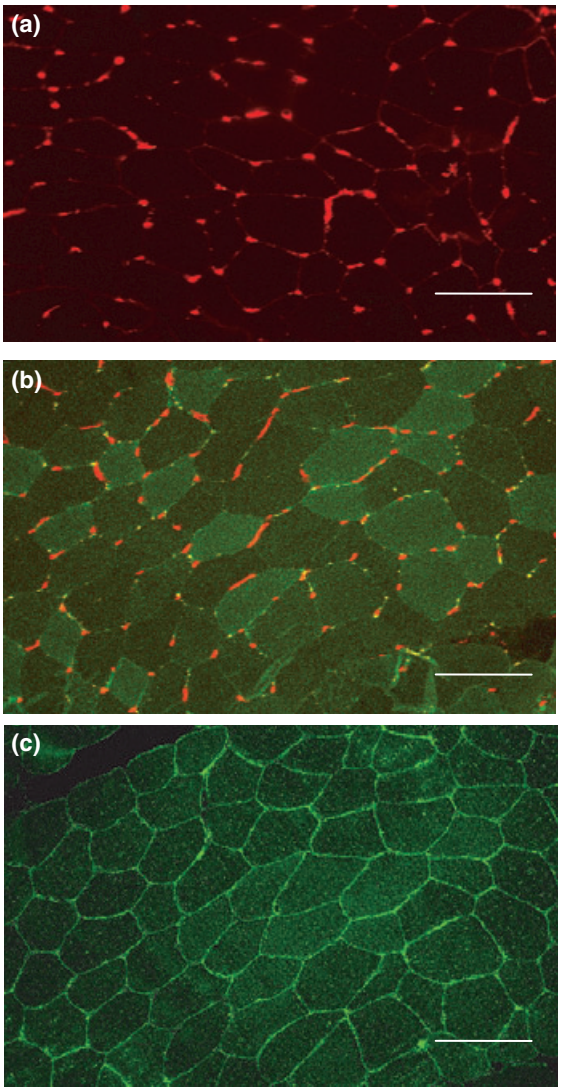

Figure 4 Immunohistochemical staining of CD36 (a), FABPpm (b), and FATP1 (c) in a muscle cross-section. Horizontal bar represents 100 μm.

patients. Furthermore, in contrast to FAT/CD36 and FATP1, we show that in FABPpm mRNA and protein expression are upregulated in the endurance trained vs. sedentary men. The latter is in line with observations in young males (Kiens *et al.* 1997, 2004).

FAT/CD36

Rodent studies have shown that skeletal muscle FAT/CD36, located in the plasma membrane, forms an important mediating role in skeletal muscle LCFA uptake (Febbraio *et al.* 1999, Ibrahimi *et al.* 1999). Recently, it was reported that FAT/CD36 is also located in the mitochondrial membrane (Campbell *et al.* 2004, Bezaire *et al.* 2006, Holloway *et al.* 2006). However, the latter is difficult to verify by immunohistochemical staining, as intracellular FAT/CD36 staining is minimal (Keizer *et al.* 2004, Vistisen *et al.* 2004). FAT/CD36 knock-out mice show a substantial decline in skeletal muscle LCFA uptake (Febbraio *et al.* 1999), whereas overexpression of FAT/CD36 has been shown to enhance LCFA oxidation rates in contracting rodent muscle (Ibrahimi *et al.* 1999). More recent human studies report greater FAT/CD36 protein content in *vastus lateralis* muscle in women compared with men (Kiens *et al.* 2004). The latter could be attributed to the fact that women have a higher type 1 muscle fibre content than men (Steffensen *et al.* 2002, Vistisen *et al.* 2004), as FAT/CD36 is expressed to a greater extent in type 1 muscle fibres (Keizer *et al.* 2004, Vistisen *et al.* 2004). Furthermore, basal LCFA flux is significantly higher in women compared with men (Koutsari & Jensen 2006), which could also be responsible for greater muscle FAT/CD36 expression in women. In accordance, elevated plasma LCFA concentrations after a high fat diet have been shown to upregulate skeletal muscle FAT/CD36 mRNA (Cameron-Smith *et al.* 2003) and protein (Roepstorff *et al.* 2004) expression. As insulin resistance and/or type 2 diabetes are generally accompanied by elevated plasma LCFA availability (Roden *et al.* 1996, Pan *et al.* 1997, Bonen *et al.* 1998a, Perseghin *et al.* 1999, Kelley & Mandarino 2000, Shulmann 2000), it seems reasonable to speculate that FAT/CD36 protein expression might also be upregulated in the type 2 diabetes state. However, we did not observe any differences in total muscle FAT/CD36 mRNA or protein content between type 2 diabetes patients and matched sedentary controls, which is in accordance with previous studies (Bruce *et al.* 2003, Bonen *et al.* 2004). We extend on these previous findings by the assessment of FAT/CD36 protein expression in muscle tissue taken from age-matched, endurance-trained controls. But even in these active males, no differences in FAT/CD36 mRNA or protein expression were evident (Fig. 1). The latter is in line with previous cross-sectional studies (Bruce *et al.* 2003, Kiens *et al.* 2004). However, it should be noted that studies investigating more acute or short-term responses to exercise have reported either an increase in FAT/CD36 mRNA expression after 90 min of exercise (Kiens *et al.* 2004) or an increase in FAT/CD36 mRNA and protein content after 8 days of training (Tunstall *et al.* 2002). FAT/CD36 therefore likely forms a more temporary adaptive response to allow greater fat utilization, but does not seem to result in a structural increase in skeletal muscle FAT/CD36 protein content (Kiens *et al.* 2004).

FABPpm

FABPpm has been suggested to be co-localized with FAT/CD36 on the sarcolemma and functions as an important LCFA transport protein (Bonen *et al.* 2002). In rat

 doi: 10.1111/j.1748-1716.2007.01698.x

muscle, fasting has been shown to upregulate sarcolemmal FABPpm protein expression (Turcotte *et al.* 1997). Human studies have reported greater FABPpm protein content in *vastus lateralis* muscle in obese men and women (Simoneau *et al.* 1999), type 2 diabetes patients (Bruce *et al.* 2003) or long-term high fat fed subjects (Roepstorff *et al.* 2004) vs. sedentary controls. In the present study, we show no differences in muscle FABPpm protein content between overweight, type 2 diabetes patients and matched sedentary controls. However, Bruce *et al.* (2003) showed significant lower FABPpm expression in weight-matched elderly controls vs. type 2 diabetes patients and young controls. In the present study, we show a significant upregulation of FABPpm mRNA and protein expression in the endurance trained males. The latter tends to be in line with previous work (Kiens *et al.* 1997, Tunstall *et al.* 2002) and implies that FABPpm content is an important factor in allowing greater LCFA oxidation in the endurance trained state.

FATP1

Although initially FATP1 was merely thought to function as very-long chain fatty acyl-CoA synthase (Coe *et al.* 1999), Wu *et al.* (2006) recently reported that FATP1 also acts as an insulin-sensitive LCFA transporter involved in diet-induced obesity in rodents. Skeletal muscle of FATP1-null mice showed reduced insulin-stimulated LCFA uptake (Wu *et al.* 2006). Therefore, we also assessed FATP1 mRNA and protein expression in skeletal muscle tissue in type 2 diabetes patients, sedentary controls and age-matched, endurance trained men. We did not detect any differences in muscle FATP1 expression between groups. Though data on FATP1 protein expression in human muscle are scarce, FATP1 mRNA (Binnert *et al.* 2000, Kiens *et al.* 2004) or protein (Bandyopadhyay *et al.* 2006) expression have been reported to be uninfluenced by training status (Kiens *et al.* 2004) and/or the obese and/or type 2 diabetes state (Binnert *et al.* 2000, Bandyopadhyay *et al.* 2006). However, it should be noted that the latter has been speculated to be gender specific (Binnert *et al.* 2000). In accordance with Bandyopadhyay *et al.* (2006), we show that total muscle FATP1 content is not upregulated or downregulated in the type 2 diabetes and/or sedentary state. Because it is not known whether sarcolemmal FATP1 content or enzymatic activity varies in the type 2 diabetes state, it remains speculative whether FATP1 plays a major role in the aetiology of insulin resistance and/or type 2 diabetes.

Overall conclusions and perspectives

Although modulating LCFA transporter content through pharmaceutical intervention could represent an effective approach to reduce plasma FA uptake, our data indicate that variations in total LCFA transporter content do not seem responsible for metabolic impairments observed in chronic metabolic disease. Skeletal muscle FAT/CD36, FABPpm and FATP1 protein expression do not seem to be dysregulated in the type 2 diabetes and/or sedentary state. However, for a transporter to be functional it needs to be located at the sarcolemma. Interestingly all the transporters have been reported to be present both at the sarcolemma as well as in various intracellular (endosomal and mitochondrial) compartments, where they are stored (Binnert *et al.* 2000, Bonen *et al.* 2000, Campbell *et al.* 2004, Chabowski *et al.* 2005). Moreover, the three LCFA transporters have been shown to translocate from the endosomal pools to the sarcolemma to upregulate LCFA uptake in a manner similar to the translocation of GLUT4 upregulating glucose transport (Douen *et al.* 1990, Hirshman *et al.* 1990, Kennedy *et al.* 1999). In isolated cardiac myocytes (Luiken *et al.* 2003, Chabowski *et al.* 2005), perfused rat hindlimb muscle (Luiken *et al.* 2002) as well as in giant vesicles prepared from skeletal muscle (Bonen *et al.* 2000), both insulin and muscle contraction have been reported to stimulate FAT/CD36 translocation to the membrane. FABPpm translocation has been reported to be induced by muscle contraction only (Chabowski *et al.* 2005), whereas FATP1 shows an insulin induced translocation from intracellular pools to the plasma membrane in both adipocytes (Stahl *et al.* 2002) and skeletal muscle (Wu *et al.* 2006). In type 2 diabetic rat and human skeletal muscle and heart, FAT/CD36 has been reported to be permanently relocated to the sarcolemma at the expense of intracellular stores and in the absence of changes in total expression (Bonen *et al.* 2004, Koonen *et al.* 2005). As we did not observe any apparent differences in LCFA transporter mRNA and/or protein expression in skeletal muscle tissue in the sedentary and/or type 2 diabetes state, we need to address possible impairments in LCFA transporter translocation next. Until recently (Bandyopadhyay *et al.* 2006), the amount of muscle tissue needed to quantify the amount of LCFA transporter in the sarcolemma has limited the progress on the assessment of the LCFA transporter translocation.

We conclude that total skeletal muscle FAT/CD36, FABPpm and FATP1 mRNA and protein expression are not upregulated or downregulated in a sedentary and/or type 2 diabetes state, and as such, are not responsible for the impairments in fat metabolism associated with type 2 diabetes. FABPpm expression is upregulated in the endurance trained state and is likely instrumental to allow greater fatty acid oxidation rates.

Conflict of interest

There is no conflict of interest.

Journal compilation © 2007 Scandinavian Physiological Society, doi: 10.1111/j.1748-1716.2007.01698.x

We thank Dr Tandon (Otsuka Research Maryland, Bethesda, MD, USA) and Dr Calles-Escandon (SmithKlineBeecham, Miama, FL, USA) for providing the antibodies for FAT/CD36 and FABPpm, respectively. We gratefully acknowledge the enthusiastic support from the volunteers participating in this experiment and Richard Jonkers for technical assistance. This study was supported by the Netherlands Heart Foundation (grant 2002.T049) and the European Commission (Integrated Project LSHM-CT-2004-005272, Exgenesis). Joost Luiken is the recipient of a VIDI-Innovational Research Grant from the Netherlands Organization of Scientific Research (NWO-ZonMw grant 016.036.305). Jan Glatz is Netherlands Heart Foundation Professor or Cardiac Metabolism.

References

Abumrad, N., Coburn, C. & Ibrahimi, A. 1999. Membrane proteins implicated in long-chain fatty acid uptake by mammalian cells: CD36, FATP and FABPpm. *Biochim Biophys Acta* **1441**, 4–13.

Adams, J., Pratipanawatr, T., Berria, R. *et al.* 2004. Ceramide content is increased in skeletal muscle from obese insulin resistant humans. *Diabetes* **53**, 25–31.

Alberti, K. & Zimmet, P. 1998. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* **15**, 539–553.

Bandyopadhyay, G., Yu, J., Ofrecio, J. & Olefsky, J. 2006. Increased malonyl-CoA levels in muscle from obese and type 2 diabetic subjects lead to decreased fatty acid oxidation and increased lipogenesis; thiazolidinedione treatment reverses these defects. *Diabetes* **55**, 2277–2285.

Bezaire, V., Bruce, C., Heigenhauser, G. *et al.* 2006. Identification of fatty acid translocase on human skeletal muscle mitochondrial membranes: essential role in fatty acid oxidation. *Am J Physiol Endocrinol Metab* **290**, E509–E515.

Binnert, C., Koistinen, H., Martin, G. *et al.* 2000. Fatty acid transport protein-1 mRNA expression in skeletal muscle and in adipose tissue in humans. *Am J Physiol Endocrinol Metab* **279**, E1072–E1079.

Boden, G. 1996. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes* **45**, 3–10.

Bonen, A., Dyck, D. & Luiken, J. 1998a. Skeletal muscle fatty acid transport and transporters. *Adv Exp Med Biol* **441**, 193–205.

Bonen, A., Luiken, J., Liu, S. *et al.* 1998b. Palmitate transport and fatty acid transporters in red and white muscles. *Am J Physiol Endocrinol Metab* **275**, E471–E478.

Bonen, A., Miskovic, D. & Kiens, B. 1999. Fatty acid transporters (FABPpm, FAT, FATP) in human muscle. *Can J Appl Physiol* **24**, 515–523.

Bonen, A., Luiken, J., Arumugam, Y., Glatz, J. & Tandon, N. 2000. Acute regulation of fatty acid uptake involves the cellular redistribution of fatty acid translocase. *J Biol Chem* **275**, 14501–14508.

Bonen, A., Luiken, J. & Glatz, J. 2002. Regulation of fatty acid transport and membrane transporters in health and disease. *Mol Cell Biochem* **239**, 181–192.

Bonen, A., Parolin, N., Steinberg, G. *et al.* 2004. Triacylglycerol accumulation in human obesity and type 2 diabetes is associated with increased rates of skeletal muscle fatty cid transport and increased sarcolemmal FAT/CD36. *FASEB J* **18**, 1144–1146.

Booth, F. & Lees, S. 2006. Physically active subjects should be the control group. *Med Sci Sports Exerc* **38**, 405–406.

Bruce, C., Anderson, M., Carey, A. *et al.* 2003. Muscle oxidative capacity is a better predictor of insulin sensitivity than lipid status. *J Clin Endocrinol Metab* **88**, 5444–5451.

Cameron-Smith, D., Burke, L., Angus, D. *et al.* 2003. A short-term, high-fat diet up-regulates lipid metabolism and gene expression in human skeletal muscle. *Am J Clin Nutr* **77**, 313–318.

Campbell, S., Tandon, N., Woldegiorgis, G., Luiken, J., Glatz, J. & Bonen, A. 2004. A novel function for fatty acid translocase (FAT)/CD36: involvement in long chain fatty acid transfer into the mitochondria. *J Biol Chem* **279**, 36235–36241.

Chabowski, A., Coort, S., Calles-Escandon, J. *et al.* 2005. The subcellular compartment-tation of fatty acid transporters is regulated differently by insulin and by AICAR. *FEBS Lett* **579**, 2428–2432.

Coe, R., Johnston-Smith, A., Frohnert, B., Watkins, P. & Bernlohr, D. 1999. The fatty acid transport protein (FATP1) is a very long chain acyl-CoA synthetase. *J Biol Chem* **274**, 36300–36304.

Douen, A., Ramlal, T., Rastogi, S. *et al.* 1990. Exercise induces recruitment of the insulin-responsive glucose transporter. Evidence for distinct intracellular insulin- and exercise-recruitable transporter pools in skeletal muscle. *J Biol Chem* **265**, 13427–13430.

Ellis, B., Poynten, A., Lowy, A. *et al.* 2000. Long-chain acyl-CoA esters as indicators of lipid metabolism and insulin sensitivity in rat and human muscle. *Am J Physiol* **279**, E554–E560.

Febbraio, M., Abumrad, N., Hajjar, D. *et al.* 1999. A null mutation in murine CD36 reveals an important role in fatty acid and lipoprotein metabolism. *J Biol Chem* **274**, 19055–19062.

Glatz, J., Luiken, J., van Bilsen, M. & van der Vusse, G. 2002. Cellular lipid binding proteins as facilitators and regulators of lipid metabolism. *Mol Cell Biochem* **239**, 3–7.

Goodpaster, B., Kelley, D., Wing, R., Meier, A. & Thaete, F. 1999. Effects of weight loss on regional fat distribution and insulin sensitivity in obesity. *Diabetes* **48**, 839–847.

Hamilton, J., Johnson, R., Corkey, B. & Kamp, F. 2001. Fatty acid transport: the diffusion mechanism in model and biological membranes. *J Mol Neurosci* **16**, 99–108.

Hirsch, D., Stahl, A. & Lodisch, H. 1998. A family of fatty acid transporters conserved from mycobacterium to man. *Proc Natl Acad Sci USA* **95**, 8625–8629.

Hirshman, M., Goodyear, L., Wardzala, L., Horton, E. & Horton, E. 1990. Identification of an intracellular pool of glucose transporters from basal and insulin-stimulated rat skeletal muscle. *J Biol Chem* **26**, 987–991.

 doi: 10.1111/j.1748-1716.2007.01698.x

Holloway, G., Bezaire, V., Heigenhauser, G. *et al.* 2006. Mitochondrial long chain fatty acid oxidation, fatty acid translocase/CD36 content and carnitine palmitoyltransferase I activity in human skeletal muscle during aerobic exercise. *J Physiol* **571**, 201–210.

Ibrahimi, A., Bonen, A., Blinn, W. *et al.* 1999. Muscle-specific over-expression of FAT/CD36 enhances fatty acid oxidation by contracting muscle, reduces plasma triglycerides and fatty acids, and increases plasma glucose and insulin. *J Biol Chem* **274**, 26761–26766.

Itani, S., Ruderman, N., Schmieder, F. & Boden, G. 2002. Lipid induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IκB-α. *Diabetes* **51**, 2005–2011.

Jensen, M. 2002. Fatty acid oxidation in human skeletal muscle. *J Clin Invest* **110**, 1607–1609.

Keizer, H., Schaart, G., Tandon, N., Glatz, J. & Luiken, J. 2004. Subcellular immuno-localisation of fatty acid translocase (FAT/CD36) in human type-1 and type-2 skeletal muscle fibers. *Histochem Cell Biol* **121**, 101–107.

Kelley, D. & Mandarino, L. 2000. Fuel selection in human skeletal muscle in insulin resistance. A reexamination. *Diabetes* **49**, 677–683.

Kelley, D., He, J., Menshikova, E. & Ritov, V. 2002. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes* **51**, 2944–2950.

Kennedy, J., Hirshman, M., Gervino, E. *et al.* 1999. Acute exercise induces GLUT4 translocation in skeletal muscle of normal human subjects and subjects with type 2 diabetes. *Diabetes* **48**, 1192–1197.

Kiens, B. 2006. Skeletal muscle lipid metabolism in exercise and insulin resistance. *Physiol Rev* **86**, 205–243.

Kiens, B., Kristiansen, S., Jensen, P., Richter, E. & Turcotte, L. 1997. Membrane associated fatty acid binding protein (FABPpm) in human skeletal muscle is increased by endurance training. *Biochem Biophys Res Commun* **231**, 463–465.

Kiens, B., Roepstorff, C., Glatz, J. *et al.* 2004. Lipid-binding proteins and lipoprotein lipase activity in human skeletal muscle: influence of physical activity and gender. *J Apply Physiol* **97**, 1209–1218.

Koonen, D., Glatz, J., Bonen, A. & Luiken, J. 2005. Long-chain fatty acid uptake and FAT/CD36 translocation in heart and skeletal muscle. *Biochim Biophys Acta* **1736**, 163–180.

Koutsari, C. & Jensen, M. 2006. Free fatty acid metabolism in human obesity. *J Lipid Res* **47**, 1643–1650.

van Loon, L. & Goodpaster, B. 2006. Increased intramuscular lipid storage in the insulin-resistant and endurance-trained state. *Pflugers Arch* **451**, 606–616.

van Loon, L., Koopman, R., Manders, R., van der Weegen, W., van Kranenburg, G. & Keizer, H. 2004. Intramyocellular lipid content in type 2 diabetes patients compared to overweight sedentary men and highly trained endurance athletes. *Am J Physiol* **287**, E558–E565.

Luiken, J., Arumugan, Y., Dyck, D. *et al.* 2001. Increased rates of fatty acid uptake and plasmalemmal fatty acid transporters in obese Zucker rats. *J Biol Chem* **276**, 40567–40573.

Luiken, J., Dyck, D., Han, X. *et al.* 2002. Insulin induces the translocation of the fatty acid transporter FAT/CD36 to the plasma membrane. *Am J Physiol Endocrinol Metab* **28**, E491–E495.

Luiken, J., Coort, S., Willems, J. *et al.* 2003. Contraction-induced fatty acid translocase/CD36 translocation in rat cardiac myocytes is mediated through AMP-activated protein kinase signaling. *Diabetes* **52**, 1627–1634.

Pan, D., Lillioja, S., Kriketos, A. *et al.* 1997. Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes* **46**, 983–988.

Perseghin, G., Scifo, P., De Cobelli, F. *et al.* 1999. Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a 1H-13C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. *Diabetes* **48**, 1600–1606.

Pohl, J., Ring, A., Hermann, T. & Stremmel, W. 2004. Role of FATP in parenchymal cell fatty acid uptake. *Biochim Biophys Acta* **1686**, 1–6.

Report of the Expert Committee 2003. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* **26** (Suppl. 1), S5–S20.

Roden, M., Price, T., Perseghin, G. *et al.* 1996. Mechanism of free fatty acid-induced insulin resistance in humans. *J Clin Invest* **97**, 2859–2865.

Roepstorff, C., Helge, J., Vistisen, B. & Kiens, B. 2004. Studies of plasma membrane fatty acid-binding protein and other lipid-binding proteins in human skeletal muscle. *Prot Nutr Soc* **63**, 239–244.

Schaffer, J. & Lodisch, H. 1994. Expression cloning and characterization of a novel adipocyte long chain fatty acid transport protein. *Cell* **79**, 427–436.

Shulmann, G. 2000. Cellular mechanisms of insulin resistance. *J Clin Invest* **106**, 171–176.

Simoneau, J., Veerkamp, J., Turcotte, L. & Kelly, D. 1999. Markers of capacity to utilize fatty acids in human skeletal muscle; relation to insulin resistance and obesity and effects of weight loss. *FASEB J* **13**, 2051–2060.

Siri, W. 1956. The gross composition of the body. *Adv Biol Med Phys* **4**, 239–280.

Stahl, A., Evans, J., Pattel, S., Hirsch, D. & Lodish, H. 2002. Insulin causes fatty acid transport protein translocation and enhanced fatty acid uptake in adipocytes. *Dev Cell* **2**, 477–488.

Steffensen, C., Roepstorff, C., Madsen, M. & Kiens, B. 2002. Myocellular triacyl-glycerol breakdown in females but not in males during exercise. *Am J Physiol Endocrinol Metab* **282**, E634–E642.

Stremmel, W., Strohmeyer, G., Brochard, F., Kochwa, S. & Berk, P. 1985. Isolation and characterization of a fatty acid-binding protein in rat liver plasma membrane. *Proc Natl Acad Sci U S A* **82**, 4–8.

Stump, D., Zhou, S. & Berk, P. 1993. Comparison of plasma membrane FABP and mitochondrial isoform of aspartate aminotransferase from rat liver. *Am J Physiol Gastrointest Liver Physiol* **265**, G894–G902.

Tunstall, R., Mehan, K., Wadley, G. *et al.* 2002. Exercise training increases lipid metabolism gene expression in human skeletal muscle. *Am J Physiol Endocrinol Metab* **283**, E66–E72.

Journal compilation © 2007 Scandinavian Physiological Society, doi: 10.1111/j.1748-1716.2007.01698.x

Turcotte, L., Srivastava, A. & Chiasson, J. 1997. Fasting increases plasma membrane fatty acid-binding protein [FABP (pm)] in red skeletal muscle. *Mol Cell Biochem* **166**, 153–158.

Vistisen, B., Roepstorff, K., Roepstorff, C., Bonen, A., van Deurs, B. & Kiens, B. 2004. Sarcolemmal FAT/CD36 in human skeletal muscle colocalizes with caveolin-3 and is more abundant in type 1 than in type 2 fibers. *J Lipid Res* **45**, 603–609.

Wu, Q., Ortegon, A., Tsang, B., Doege, H., Feingold, K. & Stahl, A. 2006. FATP1 is an insulin-sensitive fatty acid transporter involved in diet-induced obesity. *Mol Cell Biol* **26**, 3455–3467.

Yu, C., Chen, Y., Cline, G. *et al.* 2002. Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidyl-inositol 3-kinase activity in muscle. *J Biol Chem* **277**, 32915–32922.

