Insulin sensitivity is independent of lipid binding protein trafficking at the plasma membrane in human skeletal muscle: effect of a 3-day, high-fat diet

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Increased caloric intake is a burden of the Western society and often associated with elevated plasma fatty acid (FA) concentrations and decreased insulin sensitivity. The transmembrane transport of FA into cells involves, in addition to a diffusional component, a protein-mediated transport, involving a group of proteins known as lipid binding proteins (LBPs) (for review, see Ref. 16). In skeletal muscle several LBPs are expressed, such as fatty acid translocase cluster of differentiation 36 (FAT/CD36) (1), plasma membrane fatty acid binding protein (FABPpm) (8, 27), and fatty acid transport protein (FATP) 1 and 4 (6, 24).

An interaction between FAT/CD36 protein content at the sarcolemma and insulin resistance has been suggested from studies in obese and diabetic rat models (5, 35) and in obese and Type 2 diabetic patients (7). It has been proposed that a permanent relocation of FAT/CD36 protein to the sarcolemma could be a significant mechanism in the development of insulin resistance by a FAT/CD36-mediated increase in plasma FA uptake and ultimately cellular intramuscular triacylglycerol (IMTG) accumulation (2, 7). In support for this, studies in rats have shown that the relocation of FAT/CD36 to the sarcolemma in oxidative muscle was correlated with increased palmitate transport (into giant sarcolemmal vesicles) (GSV) and IMTG accumulation of insulin-resistant and type 2 diabetic rats compared with their respective lean controls (5, 35). This suggests a link between FAT/CD36, IMTG and insulin resistance. However, IMTG per se is most likely not the cause of decreased insulin sensitivity but viewed as a precursor for lipid intermediates such as long-chain acyl CoA, diacylglycerol, and ceramide, which might mediate decreased insulin sensitivity (for review, see Ref. 11). Importantly, whether increased FAT/CD36 protein at the sarcolemma is an early event in the development of decreased insulin sensitivity or whether it is a consequence of chronic oversupply of calories, increased plasma FA or chronically altered metabolism is not known.

FABPpm protein expression has been shown to be increased in skeletal muscle from obese and Type 2 diabetic patients when compared with lean controls (7). In addition, when healthy, nonobese male volunteers (aged 30–40 years) consumed a high-fat (HF) diet of 62 percent of energy (E %) from fat for 4 wk, the expression of FABPpm protein in skeletal muscle was increased (48). Interestingly, the sarcolemmal content of FABPpm protein was not different between lean, obese and Type 2 diabetic patients (7), even though it has been suggested that FABPpm and FAT/CD36 colocalize at the sarcolemma (36).

Studies have shown that FATP1 and FATP4 proteins are important for long-chain FA transport in yeast (13) and for palmitate when overexpressed in rat skeletal muscle (42). FATP proteins show acyl-CoA-synthetase activity, suggesting that FATP proteins participate in the formation of fatty acyl-CoA esters from FA that are needed for FA to be metabolized (14, 51, 55, 56). Furthermore, knockout of FATP1 protects against HF diet-induced decreased insulin sensitivity in mice, possibly by reducing IMTG (29). However, the cellular localization of FATP1 and FATP4 protein in human skeletal muscle is unknown. Previously, FATP1 and FATP4 proteins were detected at the plasma membrane in GSV from rat and mouse skeletal muscle (22, 23, 39, 42) and in t-tubuli from rat skeletal muscle (53). However, recent studies using double immunofluorescence and subcellular fractionation in C2C12 cells indicated that FATP4 protein was colocalized to the endoplasmic...
induced by HF diet. A recent study, performed to test the hypothesis that the cellular localization of FATP1 and FATP4 proteins is undefined and how these proteins are affected by an increase in plasma FA has not yet been investigated in humans.

Studies have used lipid infusion (composed of unsaturated FA) to investigate the effects of acute increased plasma FA concentration and lipid accumulation in skeletal muscle on insulin sensitivity in humans (20, 45). In the present study, we have made use of a more physiological approach by having subjects consume a diet rich in fat, consisting primarily of unsaturated FA, for 3 days. By this approach we examined the effects of increased FA availability on LBPs: 1) mRNA content, 2) total protein expression, and 3) the content of sarcolemma bound LBPs and impact on whole body insulin sensitivity in healthy, male volunteers. This study is the first to investigate LBPs trafficking at the sarcolemma in healthy human skeletal muscle subjected to increased FA availability induced by HF diet.

### MATERIALS AND METHODS

**Subjects.** Seventeen male subjects were recruited to participate in the study. Subject characteristics are given in Table 1. Before volunteering, all subjects were given verbal and written information about the study and possible risks associated with participation. Written consent was obtained from each subject. The study was approved by the Copenhagen Ethics Committee (no. KF 01 261127) and conform to the code of ethics of the World Medical Association (Declaration of Helsinki II 2008). Subjects were young, healthy, moderately trained males [All subjects: age, 24.4 ± 0.7 yr; height, 1.80 ± 0.02 m; body mass, 76 ± 2 kg; body mass index, 23.5 ± 0.5 kg/m²] that participated on a regular basis (2–3 h/wk) in leisure time activities such as running, cycling, strength training, and different game sports.

**Preexperimental testing.** For determination of whole body peak oxygen uptake (VO_{2peak}) (Table 1), all subjects performed an incremental exercise test on a bicycle ergometer (Monark Ergomedic 839E, Monark, Sweden). In addition, they filled out a questionnaire regarding habitual physical activity and exercise training. For determination of body composition (Table 1), a dual-energy X-ray absorptiometry scan (DPX-QJ Lunar, Lunar Corporation) was performed after a 4-h fasting period. All subjects completed a 3-day dietary registration for determination of their habitual diet. All foods were weighed to 1-g accuracy.

**Experimental protocol.** Before the dietary interventions, the subjects arrived by bus, car, or train at the laboratory in the morning after an overnight fast. The subjects had abstained from strenuous exercise training two days preceding the experimental day and followed a controlled habitual diet based on their dietary registration. After 30 min of rest in the supine position, blood samples (10 ml) were drawn from an antecubital vein. Two biopsies were obtained from the vastus lateralis muscle under local anesthesia (Xylocaine, 20 mg/ml; AstraZeneca, Södertälje, Sweden) of the skin and fascia. Hereafter, the subjects initiated the diet intervention. After 3 days on the experimental diet, the subjects arrived at the laboratory in the morning after an overnight fast, and postexperimental blood samples and biopsies were obtained as described above.

**Diets.** The subjects were subjected to a hypercaloric [175% of energy (E %) of estimated daily energy intake] HF diet (77 E % from fat) for 3 days, and the composition of the diet is given in Table 2. A hypercaloric, HF diet was chosen to obtain as high a lipid load as possible without a loss of compliance. To control for the possible effects of the hypercaloric intake, a group of subjects were subjected to a hypercaloric (175 E % of estimated daily energy intake) high-carbohydrate (H-CHO) diet (80 E % from carbohydrate). One volunteer completed both diet interventions, and a period of >3 wk separated the two trails, during which the subject consumed the habitual diet. However, since the dietary interventions had an impact on the subject’s everyday life and to increase compliance to the study, we decided to recruit one group of volunteers and randomized them into the two diet interventions. The groups were matched on the basis of their VO_{2peak} and training history. In total, 11 volunteers completed the HF diet, and 7 volunteers completed the H-CHO diet (Table 1). In both experimental diets, the amount of energy to be consumed was individually determined from body weight and habitual physical activity level based on guidelines from World Health Organization. The HF diet consisted primarily of salmon, sausages, avocado, various nuts, cream, olive oil, and a little bread and pasta and various fruits and vegetables. The H-CHO diet consisted among other things of white bread, low fat milk, chocolate, pasta, juice, soft drinks, fish, meatballs, and various fruits and vegetables.

**Muscle biopsies.** The first muscle biopsy was immediately frozen in liquid nitrogen and stored at −80°C for subsequent analysis of mRNA content, total protein expression of LBPs, and muscle metabolites. The second biopsy was used for the preparation of GSV.

**Animals and animal experiments.** Male Wistar rats (n = 4, weight 150–200 g) (Taconic, Skensved, Denmark) were used in this experiment to investigate whether the nondetectable FATP1 and FATP4 protein in human GSV were a species phenomenon. The animals were housed in controlled conditions with a 12:12-h light-dark cycle and were fed an ad libitum standard chow diet (Brogaarden, Denmark). For experiments, all rats were fasted for 4 h and anesthetized with an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body wt). The gastrocnemius muscles from both legs were excised and used for experiments. The gastrocnemius muscles were from both legs were excised and used for experiments and Other Scientific Purposes (Council of Europe 123, France).

**Giant sarcolemmal vesicles.** The GSV were prepared from human (25) muscle biopsies or from rat gastrocnemius muscle (47), as described above.

### Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>HF</th>
<th>H-CHO</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>Age, yr</td>
<td>23.8 ± 0.9</td>
<td>25.4 ± 1.2</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.8 ± 0.0</td>
<td>1.8 ± 0.0</td>
</tr>
<tr>
<td>BM, kg</td>
<td>74.0 ± 2.3</td>
<td>79.5 ± 1.9</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>15.2 ± 2.0</td>
<td>14.1 ± 1.3</td>
</tr>
<tr>
<td>LBM, kg</td>
<td>60.2 ± 1.7</td>
<td>67.6 ± 1.9*</td>
</tr>
<tr>
<td>VO_{2peak} l/min</td>
<td>3.8 ± 0.2</td>
<td>3.8 ± 0.2</td>
</tr>
<tr>
<td>ml kg BM^{-1} min^{-1}</td>
<td>51.8 ± 1.8</td>
<td>48.1 ± 1.7</td>
</tr>
<tr>
<td>ml kg LBM^{-1} min^{-1}</td>
<td>63.5 ± 1.8</td>
<td>56.5 ± 2.1*</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. n, no. of subjects; BM, body mass; LBM, lean body mass; VO_{2peak}, peak oxygen uptake; HF, high-fat diet; H-CHO, high-carbohydrate diet. *P < 0.05 different from HF.

### Table 2. Calculated average daily dietary composition of habitual diet, high-fat (HF) diet, and high-carbohydrate (CHO) diet

<table>
<thead>
<tr>
<th></th>
<th>Habitant</th>
<th>HF</th>
<th>H-CHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat, % of energy</td>
<td>31</td>
<td>77</td>
<td>11</td>
</tr>
<tr>
<td>Saturated fat, g/day</td>
<td>32</td>
<td>110</td>
<td>13</td>
</tr>
<tr>
<td>Monounsaturated fat, g/day</td>
<td>33</td>
<td>427</td>
<td>11</td>
</tr>
<tr>
<td>Polyunsaturated fat, g/day</td>
<td>16</td>
<td>161</td>
<td>28</td>
</tr>
<tr>
<td>Protein, % of energy</td>
<td>17</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Carbohydrate, % of energy</td>
<td>50</td>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>Alcohol, % of energy</td>
<td>2</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

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Real-time PCR. The mRNA content of FAT/CD36, FABPpm, FATP1, FATP4, and β-actin was determined by real-time PCR (ABI PRISM 7900 Sequence Detection Systems; Applied Biosystems) using the fluorogenic 5'-6-carboxyfluorescein and 3'-6-carboxy-N,N,N',N'-tetramethylrhodamine-labeled. The obtained cycle threshold values reflecting the initial content of the specific transcript in the sample were converted to an arbitrary amount by using standard curves obtained from a serial dilution of a pooled sample made from all samples. The amount of a given mRNA in a given sample was normalized to the β-actin mRNA content of the given sample. Because of tissue limitations, mRNA analyses were performed in muscle samples from seven subjects in the HF group and six subjects in the H-CHO group. Importantly, the characteristics of these subjects did not differ from the characteristics of the entire cohort (data not shown).

Muscle lysates. For determination of total protein expressions in skeletal muscle, lysates were prepared. Briefly, freeze-dried and dissected muscle tissue (~5 mg) was homogenized in ice-cold buffer B (1:80 wt/vol). Homogenates were rotated end over end for 1 h at 4°C and cleared by centrifugation 20 min at 17,500 g. Protein content in the lysates and the resuspended GSV were measured using the bicinchoninic acid method (Pierce Chemical, Rockford, IL).

Western blot analysis. Total protein expression in lysates and sarcolemma protein content from GSVs of FAT/CD36, FABPpm, FATP1, FATP4, and β-actin were analyzed by SDS-PAGE and Western blot analysis. Lysates and resuspended GSV were heated for 5 min at 96°C in Laemmli’s buffer before being subjected to SDS-PAGE and semi-dry immunoblotting. PVDF membranes were incubated with primary antibodies: anti-FAT/CD36 (R&D Systems, Abingdon, UK), anti-FABPpm (kindly donated by Jan F. C. Glatz), anti-FATP1 (kindly donated by Andreas Stahl), anti-FATP4 (kindly donated by Joachim Füllekrug), anti-β-actin (Sigma-Aldrich), anti-caveolin-1 and anti-caveolin-3 (BD Transduction Laboratories, San Jose, CA), anti-COX-1 (Invitrogen, Paisley, UK), anti-DHPRα1, anti-SERCA2 (Affinity Bioreagents, Golden, CO) and anti-F-ATPase-β (Santa-Cruz Biotechnology, Santa Cruz, CA). Secondary antibodies were from Dako Cytomation (Glostrup, Denmark). Antigen-antibody complexes were visualized using enhanced chemiluminescence (ECL+, Amersham Biosciences) and quantified by a Kodak Image Station MI2000 (Kodak, Copenhagen, Denmark).

Muscle metabolites. Muscle tissue (~50 mg wet wt) was freeze-dried and dissected free of all visible adipose tissue, connective tissue, and blood under a microscope. The dissected muscle fibers were washed with buffer A and centrifuged twice at 900 g for 10 min. The supernatant was collected and washed twice with buffer A containing 10 mM EDTA and collected to a final volume of 6 ml. Percoll (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK) was added to the suspension to a final concentration of 40%. Percoll cushion (1:80 wt/vol) was prepared by centrifugation 45 min at 50,000 g. The muscle suspensions were centrifuged at 50,000 g for 45 min. The gradient interface was collected and resuspended in a buffer containing 10 mM Tris, pH 7.4. The muscle supernatant was used for subsequent analysis.

RNA isolation and reverse transcription. Total RNA was isolated from visible blood and fat, connective, and nervous tissue. The muscle tissue was cut longitudinally into thin layers (~2 mm) and incubated in buffer A (140 mM KCl and 10 mM MOPS) with 1 μl/ml PMSF and 1,500 units collagenase (type VII, Sigma-Aldrich, St. Louis, MO). The muscles were incubated 1.5 h at 34°C in a shaking water bath. The supernatant was collected and washed twice with buffer A containing 10 mM EDTA and collected to a final volume of 6 ml. The supernatant was centrifuged at 900 g for 10 min at 4°C and cleared by centrifugation 20 min at 17,500 g. The obtained cycle threshold values reflecting the initial content of the specific transcript in the sample were converted to an arbitrary amount by using standard curves obtained from a serial dilution of a pooled sample made from all samples. The amount of a given mRNA in a given sample was normalized to the β-actin mRNA content of the given sample. Because of tissue limitations, mRNA analyses were performed in muscle samples from seven subjects in the HF group and six subjects in the H-CHO group. Importantly, the characteristics of these subjects did not differ from the characteristics of the entire cohort (data not shown).

Muscle glycojen.

**Fig. 1.** Intramuscular triacylglycerol content in vastus lateralis muscle before (open bars) and after (solid bars) 3 days of a high-fat diet (HF) (A) or a high carbohydrate diet (H-CHO) (B). Data are expressed as means ± SE. #P < 0.01 different from before HF using Wilcoxon signed rank test.

**Fig. 2.** Muscle glycogen content in vastus lateralis muscle before (open bars) and after (solid bars) 3 days of a HF (A) or H-CHO (B) diet. Data are expressed as means ± SE. *P < 0.05 different from before within diet group.
pooled and then divided into subpools for the respective analysis of intramuscular triacylglycerol and muscle glycogen.

The content of IMTG was determined as described previously (28, 54). In short, ~1 mg of the freeze-dried, dissected tissue was incubated overnight in tetraethyammoniumhydroxide. After the incubation, 3 M perchloric acid was added, and the samples were centrifuged at 22°C for 10 min at 3,000 g. The samples were neutralized with 2 M KHCO₃, and the content of glycerol was then analyzed fluorometrically.

The muscle glycogen content was determined as glucose units after acid hydrolysis of ~1 mg freeze-dried and dissected muscle tissue using a fluorometric method described by Lowry and Passonneau (34).

Blood analysis. Plasma concentrations of insulin were determined by ELISA assay (DakoCyto/insulin ELISA kit; Dako). Using colorimetric commercial assay kits, we measured plasma concentrations of FA [NEFA-HR (2) kit, Wako Chemicals, Germany], TG [triaclyglycerol GPO-PAP kit (Roche, Basel, Switzerland)] and glucose (gluco-quant kit; Roche) were measured on a Hitachi 912 automatic analyzer.

Calculations. For calculations of the habitual dietary composition and energy intake and the experimental diets, a PC program was used (Dankost3000, Danish Catering Center, Copenhagen, Denmark). Dietary compositions are given in Table 2.

The homeostatic model assessment for insulin resistance (HOMA_R) was calculated according to the following equation: [fasting plasma glucose (mmol/l)·fasting plasma insulin (μU/ml)]/22.5 (38).

**RESULTS**

Characteristics of the subjects are given in Table 1. There was no difference in body fat percentage and subjects did not change their body weight during the three days of diet interventions (HF: 74.0 ± 2.3 vs. 73.9 ± 2.2 kg; H-CHO: 79.3 ± 2.0 vs. 79.3 ± 2.0 kg). Subjects were matched on the basis of their VO₂peak. However, the subjects in the H-CHO group had an increased lean body mass (LBM) compared with the subjects in the HF group (P < 0.05) (Table 1). This led to a decreased VO₂peak per LBM in the H-CHO group compared with the HF group (P < 0.05) (Table 1).

**Diets.** The habitual dietary intake comprised 31 E% of fat, whereas the HF and H-CHO diets consisted of 77 and 11 E% of fat, respectively. The experimental HF and H-CHO diets consisted of 10 and 80 E% of carbohydrates, respectively, compared with 50 E% in the habitual diet (Table 2). The HF diet had a 2.4-, 11.9-, and 9.1-fold increased amount of saturated, monounsaturated, and polyunsaturated FA, respectively, compared with the habitual diet (Table 2).

**Intramuscular triacylglycerol and muscle glycogen.** Intramuscular triacylglycerol (IMTG) content increased from 30.6 ± 3.3 to 52.3 ± 6.8 mmol/kg dry wt (+72%; P < 0.01) after the HF diet with no change in IMTG after the H-CHO diet (Fig. 1, A and B, respectively). The content of muscle glycogen decreased from 435 ± 36 to 336 ± 24 mmol/kg dry wt (−23%; P < 0.05) after the HF diet and increased after the H-CHO diet from 487 ± 20 to 628 ± 43 mmol/kg dry wt (+29%; P < 0.05) (Fig. 2, A and B, respectively).

**Plasma measurements.** Plasma concentrations of FA increased nonsignificantly from 329 ± 38 to 469 ± 77 μmol/l (42%; P = 0.07) after the HF diet but decreased nonsignificantly from 328 ± 100 to 113 ± 21 (P = 0.08) after the H-CHO diet (Table 3). Insulin concentrations in plasma increased significantly by 54% from 4.4 ± 0.5 μU/ml to 6.8 ± 1.0 μU/ml (P < 0.05) in the HF group but not in the H-CHO group (4.1 ± 0.4 vs. 6.3 ± 1.1 μU/ml) (Table 3). Glucose concentrations in plasma were unchanged after both diets (HF: 5.0 ± 0.1 vs. 5.1 ± 0.2 mmol/l; H-CHO: 5.1 ± 0.1 vs. 5.0 ± 0.1 mmol/l). The increase in plasma insulin concentrations resulted in increased HOMA_R values from 1.0 ± 0.1 to 1.6 ± 0.3 (P < 0.05) in the HF group, indicating decreased whole body insulin sensitivity.

### Table 3. Venous plasma concentrations and HOMA_R-Index before and after 3 days of high-fat diet or high-carbohydrate diet

<table>
<thead>
<tr>
<th></th>
<th>HF Before</th>
<th>HF After</th>
<th>H-CHO Before</th>
<th>H-CHO After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma FA concentration, μmol/l</td>
<td>329 ± 38</td>
<td>469 ± 77**</td>
<td>328 ± 100</td>
<td>113 ± 21#</td>
</tr>
<tr>
<td>Plasma insulin concentration, μU/ml</td>
<td>4.4 ± 0.5</td>
<td>6.8 ± 1.0*</td>
<td>4.1 ± 0.4</td>
<td>6.3 ± 1.1</td>
</tr>
<tr>
<td>HOMA_R-Index</td>
<td>1.0 ± 0.1</td>
<td>1.6 ± 0.3*</td>
<td>0.9 ± 0.1</td>
<td>1.4 ± 0.3</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. FA, free fatty acids. *P < 0.05 different from before HF. **P = 0.07 different from before HF, #P = 0.08 different from before H-CHO using Wilcoxon signed rank test.

Fig. 3. Representative Western blots from the characterization of giant sarcolemma vesicles (GSV) from human muscle compared with lysate. F1-ATPase-β, β-subunit of the F1-ATPase; SERCA2, sarcomplasmic endoplasmic reticulum ATPase-2; DHPRa1, dihydropyridine receptor α1; COX-1, cytochrome c oxidase-1; Cav-1, caveolin-1, a protein marker of endothelial and adipocyte membrane; Cav-3, Caveolin-3, a protein marker of the sarcolemma.

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body insulin sensitivity after the subjects consumed the HF diet (Table 3). No significant change in the HOMAIR values after the H-CHO diet was observed (Table 3). Plasma TG concentrations were unchanged after both diets (HF: 0.74 ± 0.04 vs. 0.84 ± 0.12 mmol/l; H-CHO: 1.20 ± 0.16 vs. 1.86 ± 0.33 mmol/l).

Characterization of GSV from human skeletal muscle. The GSV preparations were purified plasma membrane fractions without contamination from other cellular membrane fraction as it was devoid of marker proteins of different membrane compartments analyzed by Western blotting (Fig. 3). β-Subunit of the F1-ATPase (F1-ATPase-β) and cytochrome c oxidase (COX-1) were used as markers of the mitochondrial membrane. Sarcoplasmic endoplasmic reticulum calcium ATPase-2 (SERCA2) and dihydropyridine receptor α1 (DHPRα1) were used as markers of the sarcoplasmic endoplasmic reticulum and the t-tubuli, respectively. Caveolin-1 (Cav-1) was used as a marker of endothelial and adipocyte membrane. Caveolin-3 (Cav-3) served as a positive marker of the plasma membrane. As in humans, we did not detect FATP1 or FATP4 proteins in GSV from rat skeletal muscle (Fig. 5).

mRNA content. The mRNA content of FAT/CD36 and FABPpm increased by 74% and 76%, respectively, after the HF diet (P < 0.05) (Fig. 6, A and C), whereas there was no change in the FAT/CD36 and FABPpm mRNA content after the H-CHO diet (Fig. 6, B and D). No change in FATP1 or FATP4 mRNA content was observed in the two diet groups (Fig. 6, E–H).

DISCUSSION

In obese women and Type 2 diabetic patients, an increased content of FAT/CD36 protein at the sarcolemma has been observed (7, 32), independent of changes in total FAT/CD36 protein expression. Consequently, a permanent relocation of FAT/CD36 protein from intracellular compartments to the sarcolemma was suggested. This could potentially lead to muscle insulin resistance, due to an increased plasma FA uptake and accumulation of IMTG and lipid intermediates in

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**Fig. 4.** Protein expressions in lysates from the human vastus lateralis muscle before (open bars) and (solid bars) after 3 days of a HF (A, C, E, and G) or H-CHO (B, D, F, and H) diet. Data are expressed as means ± SE. FAT/CD36 (A and B), FABPpm (C and D), FATP1 (E and F), FATP4 (G and H), and representative Western blots (I).
skeletal muscle. In the present study, we demonstrated that 3 days of HF diet induced a decrease in whole body insulin sensitivity and despite an increased IMTG content and a trend toward an increased plasma FA concentrations, this was not associated with an increased basal sarcolemmal content of FAT/CD36 protein. On the other hand, an increase in FABPpm and FAT/CD36 mRNA content was observed after consuming the HF diet in the present study. The latter supports previous findings by others (4, 9). It is well known that FA, and, in particular, unsaturated FA (18, 30, 41), are ligands or ligand precursors for the transcription factors peroxisome proliferator-activated receptors (PPARs) (17). Even though little evidence is available, reports indirectly suggest that FAT/CD36 (50) and FABPpm (3) in skeletal muscle and FATP in the heart (26) are under transcriptional control of PPARs. In the present study, there was a several-fold higher content of monounsaturated (11.9-fold) and polyunsaturated (9.1-fold) FA than of saturated FA (2.4-fold) in the HF diet compared with the habitual diet. Because no upregulation was observed in FAT/CD36 and FABPpm mRNA in response to the H-CHO diet, the present study shows that FAT/CD36 and FABPpm mRNA are upregulated in human skeletal muscle by a high availability of monounsaturated and polyunsaturated FA. However, the upregulation of FAT/CD36 and FABPpm mRNA did not translate into changes in FAT/CD36 or FABPpm protein expression after 3 days of HF diet. Indeed, some previous studies, using longer diet interventions (5 days and 4 wk) applied to human subjects, showed an increased total FAT/CD36 protein expression (>65 % of fat and 62 % of fat, respectively) (9, 49). On the contrary, one study showed no change in FAT/CD36 protein expression (1 wk, 60 % of fat) (52). The discrepancies between studies could be due to the type of FA (monounsaturated, polyunsaturated, and saturated FA) used in the diets, which were not reported in those studies (9, 49, 52). Taken together, these observations suggest that the duration of the elevated FA availability (consisting primarily of unsaturated FA) is an important factor for transcriptional activation and translational modification of FAT/CD36 and FABPpm in human skeletal muscle.

The total FAT/CD36 protein expression has previously been shown to be similar in lean, obese subjects, and in Type 2 diabetic patients (7). However, the functional pool of FAT/CD36 and FABPpm protein seemed to be allocated to the sarcolemma, because the upregulation and downregulation of the sarcolemmal pool correlated with FA transport in GSV from chronically electrically stimulated or denervated muscles (31). Indeed, FAT/CD36 protein has been shown to be permanently relocated to the sarcolemma in obese subjects and Type 2 diabetic patients when compared with lean individuals (7), and this was correlated with an increased basal FA transport into GSV and IMTG accumulation in skeletal muscle (7). A similar observation has been made in a study from the same...
increased IMTG upon short-term HF diet in humans are still elusive. Since we did not find evidence of increased sarcolemmal FAT/CD36 and FABPpm content, it could be speculated that the capacity of FAT/CD36 and FABPpm was sufficient to drive the increased FA transport and that other factors were important for the incorporation of FA into IMTG during HF conditions. Interestingly, recent studies in rodents (37) and cell models (44) suggest that lipid droplet-coating proteins are critically important for TG accumulation and degradation. It is unknown whether this is apparent in human muscle.

Together, the present findings suggest that a permanent relocation of FAT/CD36 or FABPpm to the sarcolemma under basal conditions, as observed in obese subjects and Type 2 diabetic patients (7) and in obese and diabetic rats (5, 35), is a time-dependent process beyond 3 days, both in the regulation of total FAT/CD36 and FABPpm protein expression in skeletal muscle and in the cellular location of these proteins. Importantly, from the present study, it can be concluded 1) that FAT/CD36 and FABPpm relocation to the sarcolemma do not seem to be linked to short-term, HF diet-induced decreased insulin sensitivity in humans and 2) that increased FA flux (judged by an increased IMTG) into healthy skeletal muscle during HF diet is independent of such a relocation.

In contrast to FAT/CD36 and FABPpm, FATP1 and FATP4 proteins have not yet been investigated in human skeletal muscle during HF consumption. Previous studies have shown that FATP1 and FATP4 proteins are important for long-chain fatty acid transport in yeast (13) but also in rat skeletal muscle laboratory using obese Zucker rats and their lean controls (35). Furthermore, when young (6 wk, insulin resistant) and old (24 wk, diabetic) Zucker diabetic fatty rats were compared, an association between the progression of insulin resistance, the accumulation of IMTG, and the increase in sarcolemma FAT/CD36 protein was made (5). In the present study, we did not observe any change in the sarcolemma FAT/CD36 or FABPpm protein content in human skeletal muscle, despite an increased FA uptake, as suggested by increased IMTG content. Even though IMTG accumulation per se is not a critical factor in the development of insulin resistance (19), an unfavorable increased FA transport would likely also induce accumulation of lipid intermediates known to be important factors in regulation of insulin signaling in skeletal muscle (11). Acute lipid infusion with Intralipid, primarily consisting of unsaturated FA, induced a decrease in whole body insulin sensitivity (20, 21, 43) and leg glucose uptake (20), however, not always consistent with impaired canonical insulin signaling (20). This suggests other mechanisms for lipid-induced decreased insulin sensitivity. Possibly, increased lactate production during lipid infusion (20) could indicate reduced pyruvate dehydrogenase complex activity and thereby a decreased conversion of pyruvate to acetyl-CoA, ultimately leading to decreased glucose oxidation and uptake (20). This might explain the lipid-induced decreased insulin sensitivity observed in the present study.

From this study, it appears that the mechanisms behind increased IMTG upon short-term HF diet in humans are still elusive. Since we did not find evidence of increased sarcolemmal FAT/CD36 and FABPpm content, it could be speculated that the capacity of FAT/CD36 and FABPpm was sufficient to drive the increased FA transport and that other factors were important for the incorporation of FA into IMTG during HF conditions. Interestingly, recent studies in rodents (37) and cell models (44) suggest that lipid droplet-coating proteins are critically important for TG accumulation and degradation. It is unknown whether this is apparent in human muscle.
muscle. This could indicate that these proteins are located in CD36 and FABPpm proteins. Moreover, we did not detect proteins, were upregulated by an increased FA availability, event. However, FAT/CD36 and FABPpm mRNA, but not the intracellular metabolism and handling of lipids rather than a direct cellular membrane transport.

In conclusion, the present study showed that HF diet-induced decrease in insulin sensitivity was independent of such an event. However, FAT/CD36 and FABPpm mRNA, but not the proteins, were upregulated by an increased FA availability, suggesting a time dependency in the upregulation of FAT/CD36 and FABPpm proteins. Moreover, we did not detect FATP1 and FATP4 protein in GSV from rat skeletal muscle. However, like in human skeletal muscle, we did not detect FATP1 or FATP4 protein in the sarcolemma obtained from human skeletal muscle in the basal, fasting state either before or after the HF or the H-CHO diets. As FATP1 and FATP4 proteins were previously detected in sarcolemma of rodents (22, 23, 39, 42) and in t-tubuli from rat muscle (53), we would verify that the lack of FATP1 and FATP4 in human sarcolemma was not a species phenomenon. For this purpose, we isolated GSV from rat skeletal muscle. However, like in human skeletal muscle, we did not detect FATP1 or FATP4 protein in rat sarcolemma in the basal, nonstimulated state. The discrepancies between previous findings (22, 23, 39, 42) and our observations are difficult to explain but could be explained by differences in method of preparation or different antibodies used against the FATP1 and FATP4 proteins. We have used antibodies that have been tested against knockout tissue by others: FATP1 (57) and FATP4 (40). Furthermore, in support of the present findings, Digel et al. (12) demonstrated, by using double labeling immunofluorescence microscopy and subcellular fractionation of C2C12 cells, that FATP4 was colocalized to the endoplasmic reticulum, clearly distinct from the plasma membrane. Importantly, neither differentiation into myotubes nor insulin treatment changed the localization of FATP4 (12). In addition, when human myotubes was transduced with GFP-tagged FATP1; FATP1 was localized in the cytoplasm and colocalized with the Golgi system marker GM-130 (15). Hence, the localization of FATP1 and FATP4 protein in skeletal muscle is still elusive. Studies have demonstrated that the FATP proteins show acyl-CoA-synthetase activity, suggesting that FATP proteins participate in the formation of fatty acyl-CoA esters from FA that are needed for FA to be metabolized (14, 51, 55, 56). Indeed, Lobo et al. (33) showed that FATP proteins were crucial in intracellular recycling of lipolytically generated FA, hydrolyzed from TG, into reesterification processes in adipocytes (33). Taken together, it may be speculated that the FATP proteins are more important for intracellular metabolism and handling of lipids rather than a direct cellular membrane transport.

In conclusion, the present study showed that HF diet-induced decrease in insulin sensitivity was independent of a relocation or up-regulation of FAT/CD36 or FABPpm protein at the sarcolemma and that the accumulation of IMTG content after a 3-day, unsaturated, HF diet was independent of such an event. However, FAT/CD36 and FABPpm mRNA, but not the proteins, were upregulated by an increased FA availability, suggesting a time dependency in the upregulation of FAT/CD36 and FABPpm proteins. Moreover, we did not detect FATP1 and FATP4 protein in GSV from rat or human skeletal muscle. This could indicate that these proteins are located in intracellular compartments and not at the sarcolemma in human skeletal muscle.

Perspectives and Significance

At present, little is known about LBP in human skeletal muscle, but from animal and cell models, it is evident that intact LBP function in muscle is crucial in maintaining whole body lipid homeostasis, thus preventing dyslipidemia and insulin resistance. In the present study, we sought to shed light on the involvement of LBP trafficking in human skeletal muscle during elevated plasma FA concentrations, as often seen in obese and insulin-resistant patients. Understanding localization and time dependency in the regulation and trafficking of LBP in human skeletal muscle is important to elucidate some of the mechanisms behind the etiology of obesity and insulin resistance. Future work should focus on the regulatory mechanisms underlying LBP trafficking in skeletal muscle.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: A.B.J., B.K., and J.J. conception and design of research; A.B.J., K.K., and J.J. performed experiments; A.B.J., A.K.S., and H.P. analyzed data; A.B.J., B.K., and J.J. interpreted results of experiments; A.B.J. prepared figures; A.B.J. drafted manuscript; A.B.J., B.K., and J.J. edited and revised manuscript; A.B.J., A.K.S., K.K., H.P., B.K., and J.J. approved final version of manuscript.

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