High insulin levels are required for FAT/CD36 plasma membrane translocation and enhanced fatty acid uptake in obese Zucker rat hepatocytes

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Submitted 20 December 2011; accepted in final form 29 May 2012

Buqué X, Cano A, Miquilena-Colina ME, García-Monzón C, Ochoa B, Aspichueta P. High insulin levels are required for FAT/CD36 plasma membrane translocation and enhanced fatty acid uptake in obese Zucker rat hepatocytes. Am J Physiol Endocrinol Metab 303: E504–E514, 2012. —In hepatocytes and adipocytes, insulin increases fatty acid translocase (FAT)/CD36 translocation to the plasma membrane (PM), enhancing fatty acid (FA) uptake. Evidence links increased hepatic FAT/CD36 protein amount and gene expression with hyperinsulinemia in animal models and patients with fatty liver, but whether insulin regulates FAT/CD36 expression, amount, distribution, and function in hepatocytes is currently unknown. To investigate this, FAT/CD36 protein content in isolated hepatocytes, subfractions of organelles, and density-gradient isolated membrane subfractions was analyzed in obese and lean Zucker rats by Western blotting in liver sections by immunohistochemistry and in hepatocytes by immunocytochemistry. The uptake of oleate and oleate incorporation into lipids were assessed in hepatocytes at short time points (30–600 s). We found that FAT/CD36 protein amount at the PM was higher in hepatocytes from obese rats than from lean controls. In obese rat hepatocytes, decreased cytoplasmic content of FAT/CD36 and redistribution from low- to middle- to high-density subfractions of microsomes were found. Hallmarks of obese Zucker rat hepatocytes were increased amount of FAT/CD36 protein at the PM and enhanced FA uptake and incorporation into triglycerides, which were maintained only when exposed to hyperinsulenic conditions (80 mU/l). In conclusion, high insulin levels are required for FAT/CD36 translocation to the PM in obese rat hepatocytes to enhance FA uptake and triglyceride synthesis. These results suggest that the hyperinsulinemia found in animal models and patients with insulin resistance and fatty liver might contribute to liver fat accumulation by inducing FAT/CD36 functional presence at the PM of hepatocytes.

fatty acid translocase; steatosis; hyperinsulinemia; free fatty acid; nonalcoholic fatty liver disease; triglyceride

FATTY ACID TRANSLOCASE (FAT)/CD36 has been recognized as the most important protein implicated in fatty acid (FA) uptake by skeletal muscle cells, cardiomyocytes, and adipocytes (36, 8, 16), being required at the plasma membrane (PM) for its function as FA transporter. In these cell types, FAT/CD36 cellular distribution is regulated by muscle contraction and/or insulin, which induce protein translocation to the PM from an intracellular pool (19, 31, 39). This particular regulation allows these tissues to respond to acute and chronic metabolic requirements, turning FAT/CD36 into a key regulator of whole body lipid homeostasis (8). In certain disorders characterized by defective response to insulin action in muscular and adipose tissues, such as obesity and type 2 diabetes, when FAT/CD36 protein is increased at the PM, FA uptake also increases (19, 5, 35), leading to unbalanced energetic metabolism and intracellular lipid accumulation (8, 42). The lipid storage could, in turn, cause the aggravation of peripheral insulin resistance by interfering with hormone intracellular signaling (15).

The liver plays an important role in FA metabolism and flux through different tissues. To avoid lipotoxicity of FA excess within the cells, hepatocytes transform the captured FA into triglycerides (TG), which are released in very-low-density lipoproteins to peripheral tissues (18). FAT/CD36 expression is normally weak in hepatocytes (4, 28), but we and others have demonstrated that FAT/CD36 gene expression and protein amount increased concomitantly with hepatic TG content in different models of hepatic steatosis (9, 13, 24). Further studies showed that FAT/CD36 is a common target of lipogenic genes, the upregulation of which promotes hepatic steatosis in a murine model (44). In addition, Bechmann et al. (3) found a significant correlation between FAT/CD36 mRNA and apoptosis in patients with nonalcoholic steatohepatitis. Interestingly, FAT/CD36 protein presence at the PM of hepatocytes positively correlated with plasma insulin levels in nonalcoholic fatty liver disease (NAFLD) patients and in hepatitis C virus (HCV)-infected patients with concomitant fatty liver (30), but whether insulin might influence FAT/CD36 protein content, redistribution, and function in hepatocytes is currently unknown, and experimental studies are needed to prove this hypothesis.

To this end, the aims of the present study were to investigate the effect of insulin on modulation of FAT/CD36 gene expression and protein amount in murine hepatocytes and to analyze whether the insulin-driven FAT/CD36 redistribution contributes to enhance FA uptake in hepatocytes.

MATERIALS AND METHODS

Animals. Six-, nine-, and twelve-week-old male obese Zucker (OZ) rats, age-matched lean Zucker (LZ) rats, and 10-wk-old male C57BL/6J mice were purchased from Charles River Laboratory (Barcelona, Spain) and housed under a 12:12-h light-dark cycle with ad libitum access to water and standard D04 diet (Panlab, Spain). Animals were food deprived for 1 h before being anesthetized (60 mg/kg pentobarbital sodium ip) and euthanized. All procedures were approved by the Basque Country University Ethics Committee in accordance with the Spanish Guidelines for the Care and Use of Laboratory Animals.

Assessment of steatosis grade. Formalin-fixed, paraffin-embedded liver sections (5 μm thick) were stained with hematoxylin and eosin

E504 0193-1849/12 Copyright © 2012 the American Physiological Society http://www.ajpendo.org
and examined by an experienced pathologist blinded to the animal groups. Hepatic steatosis was defined as the percentage of hepatocytes containing macrovesicular fat droplets. The steatosis grade was evaluated exactly as detailed before (9) in 10 randomly chosen fields (×20 magnification) from each liver specimen and graded as follows: grade 0 (<5% of hepatocytes affected), grade 1 (5–32% of hepatocytes affected), grade 2 (33–66% of hepatocytes affected), or grade 3 (>66% of hepatocytes affected).

Isolation and incubation of hepatocytes. Rat and mouse hepatocytes were isolated by perfusion with collagenase as previously described (2) and, when necessary, they were incubated at 37°C and 5% CO₂ during 4 h. Plasma insulin levels range from 25 to 30 mU/l in Sprague-Dawley rats (21, 25), from 17 to 22 mU/l in LZ rats, and from 70 to 110 mU/l in OZ rats (9). Plasma insulin levels range from 18 to 30 mU/l in C57BL/6J (23, 29) mice and increase at least 5 times from 70 to 110 mU/l in OZ rats (9). The incubation medium was supplemented with 15 or 80 mU/l insulin (Novo Nordisk) (insulatard NPH, 100 U/ml), which were, in approximately, serum insulin concentrations in control and insulin-resistant-induced C57BL/6J mice, cDNA synthesis, and quantitative real-time PCR.

RNA extraction and quantitative real-time PCR. Total RNA extraction from hepatocytes, cDNA synthesis, and quantitative real-time PCR (qPCR) were performed exactly as described previously (9). FAT/CD36 (GenBank accession nos. NM_031561.2 and Rn00580728_m1) and the housekeeping genes ribosomal 18S (GenBank accession nos. X03205.1 and Hs9999999_s1) and cyclophylin (GenBank accession nos. NM_031561.2 and Rn00580728_m1) were measured by qPCR using Taqman Gene Expression Assays (Applied Biosystems). The slides were incubated with the primary and secondary antibodies shown in Table 1 and developed with 0.5 mg/ml 3,3′-diaminobenzidine tetrahydrochloride (Sigma Chemical) and hydrogen peroxide. Liver tissue area occupied by FAT/CD36-positive cells was measured using a high-resolution digital videocamera (Nikon Dxm 1200, Tokyo, Japan) connected to a light microscope (Nikon Eclipse E400) equipped with planApocromatic ×20 ×40, and ×60 objectives (Nikon). The evaluation and image analysis procedures were performed with the AnalySIS software (Soft Imaging System, Münster, Germany). Values were obtained in six different lobular areas where hepatocytes are the predominant cell type. The average value was considered as the FAT/CD36 expression index for each liver sample and was expressed as the percentage of liver tissue area occupied by FAT/CD36-expressing cells.

Immunocytochemistry. Immunocytochemistry was performed basically as described elsewhere (33). Briefly, hepatocytes were incubated on coverslips for 4 h, washed and fixed with formaldehyde, permeabilized with Triton X-100, and blocked with newborn calf serum. FAT/CD36 and subcellular markers protein amount was determined with the antibodies shown in Table 1. Staining was assessed using an Olympus Fluorview FV500 confocal microscope (Basque Country University Research Facility).

Oleate uptake and oleate incorporation into lipids. To assess FA uptake by FAT/CD36, a long-chain FA transporter, and incorporation of FA into lipids, oleic acid was used as modelized previously (16). After 2 h of adhesion, hepatocytes were incubated in the presence of 15 or 80 mU/l insulin concentrations for 4 h as described above, and then the medium was replaced with 2 ml of fresh medium containing 0.2 M oleate-BSA complex (molar ratio 2:1) and 1.6 μCi of radiolabeled [9,3H]oleic acid per plate (4 μCi/μmol specific activity). The pulse was carried out for 30, 150, and 600 s at 37°C. Oleate uptake was ended by removal of the medium and five washes with 1 ml of cold PBS. Cells were either lysed or used for lipid extraction. Lysis was performed with 1 M NaOH and the lysate put into 5 ml of scintillation cocktail. The radioactivity incorporated into hepatocytes was determined in a RACKBETA scintillation counter.

Table 1. Antibodies and conditions used in immunoassays

<table>
<thead>
<tr>
<th>Supplier</th>
<th>Antibody Description</th>
<th>Assay and Dilution</th>
<th>Dilution</th>
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<tbody>
<tr>
<td>Santa Cruz Biotechnologies</td>
<td>Goat anti-FAT/CD36</td>
<td>WB</td>
<td>1:1,000</td>
</tr>
<tr>
<td></td>
<td>Goat anti-calcregulin</td>
<td>IHC</td>
<td>1:100</td>
</tr>
<tr>
<td></td>
<td>HRP-bovine anti-goat IgG</td>
<td>WB</td>
<td>1:1,000</td>
</tr>
<tr>
<td></td>
<td>Mouse anti-FAT/CD36</td>
<td>IHC</td>
<td>1:100</td>
</tr>
<tr>
<td></td>
<td>Mouse anti-GM130</td>
<td>IHC</td>
<td>1:100</td>
</tr>
<tr>
<td></td>
<td>Mouse anti-Na-K ATPase c1</td>
<td>IHC</td>
<td>1:2,000</td>
</tr>
<tr>
<td></td>
<td>Rabbit anti-β-tubulin</td>
<td>IHC</td>
<td>1:2,000</td>
</tr>
<tr>
<td>Molecular Probes</td>
<td>Alexa Fluor 594-goat anti-rabbit IgG</td>
<td>IHC</td>
<td>1:2,000</td>
</tr>
<tr>
<td></td>
<td>Alexa Fluor 488-goat anti-mouse IgG</td>
<td>IHC</td>
<td>1:2,000</td>
</tr>
<tr>
<td></td>
<td>Alexa Fluor 488-chicken anti-goat IgG</td>
<td>IHC</td>
<td>1:2,000</td>
</tr>
<tr>
<td>Cell Signaling Technology</td>
<td>HRP-horse anti-mouse IgG</td>
<td>IHC</td>
<td>1:2,000</td>
</tr>
<tr>
<td>Dakocytomation</td>
<td>HRP-rabbit anti-goat IgG</td>
<td>IHC</td>
<td>1:2,000</td>
</tr>
<tr>
<td></td>
<td>Goat serum (negative control)</td>
<td>IHC</td>
<td>1:2,000</td>
</tr>
<tr>
<td></td>
<td>Mouse anti-CD68</td>
<td>IHC</td>
<td>1:2,000</td>
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For incorporation studies, lipids were exhaustively extracted from hepatocytes and separated by thin-layer chromatography (10). Lipid classes were visualized by exposure to iodine vapor, the bands corresponding to triglycerides and diglycerides were scraped, and the associated $^3$H was determined by scintillation counting.

Quantification of triglycerides. TG were quantified in freshly isolated hepatocytes after being exhaustively extracted and separated by thin-layer chromatography, and the amount of TG was calculated by optical densitometry as detailed elsewhere (10).

Total protein measurements. Protein concentrations were measured using commercial bicinchoninic acid reagent (Thermo Fisher Scientific, Rockford, IL).

Statistical analysis. Data are presented as means ± SD unless otherwise mentioned. Multiple comparisons among groups were statistically evaluated by two-way ANOVA followed by the Bonferroni test (see Fig. 4). Otherwise, significant differences between phenotypes and/or insulin treatments were determined by two-tailed unpaired Student’s t-test. Correlation analyses were carried out using Spearman’s r-test. Statistical analyses were performed using SPSS v. 15.0 (SPSS, Cary, NC) software with two-sided tests, with a $P$ value of <0.05 considered as statistically significant.

RESULTS

Increased FAT/CD36 protein amount at the hepatocyte PM is associated with enhanced FA uptake in OZ rats. In LZ rat liver, FAT/CD36 was restricted to sinusoidal endothelium and Kupffer cells (Fig. 1A1), in a similar way as in the liver of 6- and 9-wk-old OZ rats (Fig. 1A2). However, immunostaining showed that in OZ12 rat livers, FAT/CD36 was also at the PM of hepatocytes (Fig. 1A3 and 1A4). In addition, in serial section of OZ12 rat liver (Fig. 1A5 and 1A6), sinusoidal-lining cells express CD36 (Fig. 1A5) and CD68, a marker of Kupffer cells (Fig. 1A6).
Hepatic FAT/CD36 expression index (Fig. 1B) and total protein amount in liver (Fig. 1C) was slightly higher in OZ12 rats than in LZ12 rats, whereas no significant differences were observed in the hepatic FAT/CD36 expression index in OZ9 and OZ6 rats with respect to their age-matched LZ control littermates (Fig. 1B) nor in the FAT/CD36 protein amount (data not shown).

A positive correlation between hepatic FAT/CD36 expression index and the histological grade of steatosis ($r = 0.930$, $P < 0.001$; Fig. 2A) and serum insulin levels ($r = 0.669$, $P < 0.049$; Fig. 2B) was observed in OZ12 rats, whereas no correlation was found in LZ12 rats (Fig. 2, A and B).

To determine the effect of insulin on FAT/CD36 cellular distribution, FA uptake and esterification in OZ12 rat hepatocytes, immunocytochemistry and oleate uptake and incorporation studies were performed in OZ12 and LZ12 rat hepatocytes exposed to different insulin concentrations. In LZ12 rat hepatocytes, the incubation with 15 or 80 mU/l of insulin did not have any effect on the cellular FAT/CD36 distribution (Fig. 3, A and B) or FA uptake after 30 or 150 s of incubation; however, a slight increase was found at longer periods (Fig. 4, A and B) ($P \leq 0.01$, Bonferroni posttest).

Regarding FA incorporation into lipids, after 600 s of incubation with labeled oleate, esterification into TG (Fig. 4C) and diglycerides (Fig. 4D) was higher in LZ rat hepatocytes exposed to 80 mU/l than in those stimulated with 15 mU/l ($P < 0.001$, Bonferroni posttest).

When OZ12 rat hepatocytes were exposed to low insulin doses (15 mU/l), FAT/CD36 presence at the PM was low (Fig. 3C), and no significant increase in oleate uptake or incorporation into lipids was observed compared with LZ12 rat hepatocytes incubated with either high or low insulin concentrations (Fig. 4, C and D). In contrast, high insulin concentration (80 mU/l) induced an increase of FAT/CD36 content at the PM of OZ12 rat hepatocytes (Fig. 3D) in parallel with a significant rise in oleate uptake (Fig. 4B), being ~30% higher with respect to other rat groups at the earlier time point (Fig. 4A). After 4 h of incubation with 80 mU/l insulin, incorporation of oleate into TG (Fig. 4C) and diglycerides (Fig. 4D) in OZ12 rat hepatocytes was higher than in the other experimental groups, which is consistent with the increased hepatocyte TG content (Fig. 4E).

To assess whether wild-type hepatocytes show insulin-induced FAT/CD36 translocation similar to what has been found in myocytes, hepatocytes from C57BL/6J mice were incubated with 15 or 80 mU/l for 4 h (Fig. 5). Translocation of FAT/CD36 protein to the PM did not occur with 15 or 80 mU/l insulin (Fig. 5A). Besides, oleate uptake was not increased in C57BL/6J hepatocytes incubated with 80 mU/l insulin compared with those incubated with 15 mU/l (Fig. 5B).

FAT/CD36 translocates from internal pools to the PM in OZ rat hepatocytes. To characterize FAT/CD36 relocation in freshly isolated OZ rat hepatocytes, we quantified FAT/CD36 protein amount in nucleus-, mitochondria-, and trafficking vesicle-enriched subcellular fractions as well as in membrane-enriched subcellular fractions. FAT/CD36 content in the 100,000-g supernatant, corresponding to the vesicle-enriched fraction, of OZ12 hepatocytes was 36% lower than in LZ12 rats ($P < 0.05$; Fig. 6A), whereas no changes were observed in the 1,000-g pellet or in the 33,000-g pellet, corresponding to...
nucleus- and mitochondria-enriched fractions, respectively. Concomitantly, in the membrane-enriched fraction, FAT/CD36 was predominantly in middle-density subfractions of microsomes (F6–F9) from LZ12 hepatocytes, whereas in OZ12 hepatocytes FAT/CD36 was more abundant in high-density subfractions (F17–F18) (Fig. 6B). Such protein profile was more evident when subfractions were grouped into low- to middle- (F1–F9) and middle- to high- (F10–F18) density ones, since the amount of FAT/CD36 was significantly lower (10-fold) in low- to middle-density subfractions of microsomes from OZ12 rat hepatocytes than in those from LZ12 rat hepatocytes (P < 0.001), and conversely, the FAT/CD36 protein content was significantly higher (3-fold) in middle- to high-density microsomes from OZ12 rat hepatocytes compared with those from LZ12 rat hepatocytes (P < 0.001) (Fig. 6C). Changes in FAT/CD36 protein profile were not associated with significant changes in the subcellular distribution of GM130, calregulin, or Na-K-ATPase, protein markers of cis-Golgi, endoplasmic reticulum, and PM, respectively (data not shown).

Immunocytochemical studies in primary hepatocytes showed that FAT/CD36 did not colocalize with calregulin (Fig. 6D) or caveolin-1, the caveolae marker at the PM (Fig. 6E).

Curiously, in LZ12 rat hepatocytes incubated with 15 mU/l insulin, FAT/CD36 was found close to GM130 but they did not show colocalization (Fig. 6F).

Increased FAT/CD36 protein in the PM is not associated with enhanced FAT/CD36 protein amount in OZ rat hepatocytes. Finally, to analyze whether the increased FAT/CD36 at PM in OZ12 rat hepatocytes might be due to increased protein availability, we measured protein by immunoblotting. We found that, whereas FAT/CD36 mRNA levels in OZ9 and OZ12 freshly isolated rat hepatocytes were around sixfold higher than in their lean control rats (Fig. 7A), no significant changes were observed in FAT/CD36 protein abundance compared with their controls (Fig. 7D). In addition, the increase in FAT/CD36 mRNA levels was maintained after exposure to insulin for 4 h. In fact, OZ12 rat hepatocytes stimulated with 15 or 80 mU/l insulin showed a 5.7- or 5.1-fold increase, respectively, compared with LZ12 rat hepatocytes under similar experimental conditions (Fig. 7B and C). However, when OZ12 rat hepatocytes were incubated with 80 mU/l insulin for 24 h, FAT/CD36 mRNA levels decreased, and the differences with respect to LZ12 rat hepatocytes incubated with 15 mU/l disappeared (Fig. 7B). Interestingly, the total protein amount of FAT/CD36 remained unaltered in all insulin-stimulated rat hepatocytes (Fig. 7D).

**DISCUSSION**

FA transport and use by different tissues is of high relevance for maintaining whole body lipid homeostasis. In skeletal and cardiac muscle cells, FAT/CD36 translocates to the PM in response to mechanical (muscle contraction) and hormonal (insulin) stimuli (19, 31, 39). In normal liver, FAT/CD36 is weakly expressed in hepatocytes (1, 28, 40), suggesting that...
FA uptake is largely FAT/CD36 independent under physiological conditions. However, we have recently shown that FAT/CD36 gene expression and protein content at the PM of hepatocytes is increased in NAFLD and HCV patients with fatty liver (30). In addition, in the present study we demonstrate that FAT/CD36 protein is also enhanced at the PM of OZ12 rat hepatocytes, a well-known animal model of obesity, insulin resistance, hyperinsulinemia, and hepatic steatosis (26).

Fig. 4. Oleate uptake and incorporation into triglycerides (TG) is increased in hepatocytes from OZ12 rats when incubated with high insulin concentrations. Oleate uptake by hepatocytes from LZ12 and OZ12 rats incubated for 4 h with 15 or 80 mU/l insulin. Oleate uptake into cells was measured at 30 (A), 150, and 600 (B) s after the addition of 0.2 mM oleate (4 μCi/μmol specific activity) to culture medium. Time course of oleate incorporation into TG (C) and diglycerides (D) by hepatocytes from LZ12 (○, ●) and OZ12 rats (□, ■) incubated for 4 h with 15 (○, □) or 80 mU/l insulin (●, ■) was measured after the same incubation periods. E: TG amount in LZ12 (open bar) and OZ12 (filled bar) rat hepatocytes. Values, given as nmol of oleate incorporated/mg cell protein or as μg of lipid/mg cell protein, are expressed as means ± SD; n = 4–11 rats each group. Statistical significance by ANOVA is marked in curves. Statistical significance according to Bonferroni posttest is marked** \( P < 0.01 \) and *** \( P < 0.001 \), OZ12 80 mU/l insulin vs. all other conditions; aa \( P < 0.01 \), aaa \( P < 0.001 \), OZ12 80 mU/l insulin vs. both LZ12 15 mU/l and LZ12 80 mU/l insulin.
levels, PM expression and FA uptake increase compared with LZ rat values. These results indicate that high insulin concentrations are required to maintain functional FAT/CD36 translocation to the PM of steatotic rat hepatocytes. Consistent with the increased TG accumulation in hepatocytes and the increased VLDL-TG secretion (9), we show that in the short term TG synthesis is enhanced, an increase that is even more marked when hepatocytes are incubated with high insulin concentrations, which could be due, at least in part, to the increased FA uptake. The fact that the amount of FAT/CD36 protein in hepatocytes from OZ resembled that of those from LZ rats, and that FAT/CD36 content decreased in vesicle-enriched subcellular fractions whereas it increased in membrane-enriched subcellular fractions in freshly isolated OZ12 rat hepatocytes, reinforces the idea of the translocation of FAT/CD36 to the PM. Taken together, these results provide evidence for a functional FAT/CD36 translocation to the PM in steatotic rat hepatocytes from an intracellular storage pool. To our knowledge, the molecular basis underlying FAT/CD36 relocation in hepatocytes remains to be defined. In adipocytes, insulin potently induces palmitoylation of FAT/CD36, facilitating the transport of this protein to the PM (22, 41). OZ rats have hyperinsulinemia along with increased intrahepatic FA levels and de novo FA synthesis (9); it might therefore be conceivable that insulin-mediated palmitoylation could also be involved in FAT/CD36 translocation in steatotic rat hepatocytes.

Our data show that whereas FAT/CD36 protein amount remains unaltered in OZ12 rat hepatocytes, the liver content is slightly increased. As demonstrated in this work, OZ12 rat liver contains FAT/CD36 protein in Kupffer cells. FAT/CD36 expresses in hepatocytes, endothelial cells, and Kupffer cells (7, 17, 27), so it would not be surprising that the increase in liver FAT/CD36 protein content could be due in part to steatosis-associated tissular enrichment in Kupffer cells. Bieghs and colleagues (6, 7) demonstrated that targeted inactivation of scavenger receptors A and CD36 in Kupffer cells reduced the hepatic inflammation and tissue destruction associated with nonalcoholic steatohepatitis. In a previous work (9), we demonstrated that OZ12 rats exhibited greater hepatosteatosis histological grade, liver damage, and body weight gain than OZ6 and OZ9 rats. Taken together, it could be hypothesized that the increased FAT/CD36 protein amount in OZ12 rat liver could be linked with the increased liver damage.

In the present study, we found that FAT/CD36 mRNA levels progressively increased in parallel with obesity and the hepatic steatosis setup. The results demonstrate that whereas FAT/CD36 mRNA levels increased in hepatocytes from obese Zucker rats, the total protein amount remained stable. Furthermore, when incubated with high insulin concentrations, FAT/CD36...
CD36 mRNA decreased independently of phenotype without affecting FAT/CD36 protein levels.

The lack of translation of FAT/CD36 mRNA into protein could be understood as an increase in FAT/CD36 mRNA half-life without additional translation, as a defective translation, or even as increased degradation of the newly synthesized protein. As far as we know, increased mRNA stability or defective translation has not been reported for FAT/CD36. Nevertheless, FAT/CD36 is ubiquitinated on two lysine residues on its small intracellular COOH-terminal domain (37), promoting rapid degradation of this FA transporter through the proteasome (11). Moreover, the ubiquitination process seems to be enhanced by FA (37), and FA availability should be increased in OZ12 rat hepatocytes. Thus, it could be considered that FAT/CD36 ubiquitination and degradation might also be enhanced, explaining, at least in part, the divergence observed between FAT/CD36 mRNA and protein levels in hepatocytes from OZ rats.

As far as we are aware, most of the published works demonstrate that FAT/CD36 overexpression at the PM is linked to increased FA uptake (8, 16, 19, 31, 39). Although one study carried out in FAT/CD36-deficient Chinese hamster ovary cells showed that the mere overexpression of FAT/CD36 at the PM of these cells was not sufficient to provoke an increased FA uptake (14), in the present study we provide evidence showing that, when exposed to high insulin levels, FAT/CD36 overexpressed at the PM of OZ12 rat hepatocytes and FA uptake increased specifically at the earlier time points.
Interestingly, this hepatocellular FA influx kinetic reflects protein-mediated transport and is independent of intracellular FA metabolism (38). In addition, the oleate uptake resembled that of LZ rat hepatocytes when FAT/CD36 did not overexpress at the PM of OZ12 rat hepatocytes even when LZ rat hepatocytes were exposed to normal insulin levels, an effect more marked at the earlier time points. We show that this is also true in mice, as high insulin levels did not affect FA uptake or FAT/CD36 presence at the PM of healthy mice hepatocytes. Thus, we demonstrate that insulin-driven FAT/CD36 PM expression in obese steatotic Zucker rat hepatocytes is a key factor to increasing protein-mediated transport of FA.

Noteworthy, enhanced sarcolemmal FAT/CD36 content increased FA uptake, contributing to TG storage within muscle cells in OZ rats (12, 20). Moreover, we have recently demonstrated that hepatic FAT/CD36 overexpression is significantly associated with increased steatosis in NASH and HCV patients with fatty liver (30). Interestingly, FAT/CD36 was predominantly located at the PM of hepatocytes in these chronic liver diseases, reinforcing the assumption that FAT/CD36 translocation to the PM of hepatocytes may play a relevant role in the steatosis setup not only in rodents but also in humans. In conclusion, our results demonstrate that high insulin levels are required for functional FAT/CD36 expression at the PM of obese rat hepatocytes and that insulin alone is not sufficient to induce FAT/CD36 protein translocation in LZ rat or in C57BL/6J mouse hepatocytes. Thus, these findings suggest that not only high insulin concentrations but also other factors such as increased intracellular FA availability might be the major stimuli enhancing FAT/CD36 translocation to the PM of hepatocytes. In the presence of hyperinsulinemia due to insulin resistance, a metabolic disorder that is increasing in Western countries, modulating FAT/CD36-mediated FA uptake in hepatocytes might be of relevance for the prevention/treatment of fatty liver by attenuating the potential toxic effects of excessive FA content within liver cells.

ACKNOWLEDGMENTS

We thank Montse Busto (University of the Basque Country, Bilbao, Spain) for excellent technical assistance, Servicios Generales de investigación and Unidad de Formación e Investigación UFI 11/20 from University of Basque Country UPV/EHU for technical and human support.

GRANTS

This work was supported by the Basque Government, IT-336-10 (to X. Buqué, B. Ochoa, and P. Aspichueta) and SA-2010/0025 (to X. Buqué and P. Aspichueta), by University of Basque Country UPV/EHU, UFI 11/20 (to X. Buqué and P. Aspichueta) and Instituto de Salud Carlos III, PI10/00067, and Fundación Eugenio Rodríguez Pascual (to C. García-Monzón). CIBERehd is funded by the Instituto de Salud Carlos III, Spain.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).
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