Fatty Acid Translocase (FAT/CD36) Is Localized on Insulin-Containing Granules in Human Pancreatic β-Cells and Mediates Fatty Acid Effects on Insulin Secretion

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The membrane receptor FAT/CD36 facilitates the major fraction of long-chain fatty acid (FA) uptake by muscle and adipose tissues. In line with the well-known effects of FA metabolism on carbohydrate utilization and insulin responsiveness, altered expression of CD36 has been linked to phenotypic features of the metabolic syndrome including insulin resistance and dyslipidemia. FA metabolism is also known to significantly affect insulin secretion. However, the role of CD36 in this process remains unknown, since its expression levels and function in the pancreas have not been explored. In the present study, freshly isolated human islets and a mouse-derived β-cell line (MIN6) were shown positive for CD36 expression by RT-PCR, Western blot, and immunofluorescence. The identity of the PCR product was confirmed by microsequencing. The identified transcript was translated and the protein was expressed and subjected to the known posttranslational glycosylation. Fluorescence resonance energy transfer analysis and subcellular protein fractionation indicated that insulin and CD36 are colocalized in the secretory granules of β-cells. Islet CD36 functioning in FA uptake because this process was blocked by the irreversibly CD36 inhibitor sulfoconjugimidyl-oleate. More importantly, sulfoconjugimidyl-oleate reversed enhancing and inhibiting effects, respectively, of acute and long-term palmitate incubations on glucose-dependent insulin secretion. In conclusion, our study demonstrates that human islets express CD36 in the plasma membrane as well as in the insulin secretory granules. CD36 activity appears important for uptake of FA into β-cells as well as for mediating their modulatory effects on insulin secretion.

Chronic elevation of plasma fatty acid (FA) levels is a determinant of insulin resistance (1–4). Various studies have demonstrated that high-fat feeding or intravenous infusion of lipid emulsions, which raise circulating free fatty acid (FFA) levels, is associated with a decreased insulin-stimulated glucose uptake (5–12). FAs cause insulin resistance by impairing activation of the intracellular signaling molecules that mediate the action of insulin in various tissues (5,10). In line with this, improvements in FA uptake and utilization appear to play an important role in the insulin-sensitizing effects of a relatively new class of antidiabetic drugs termed thiazolidinediones (13–20).

FA uptake consists of two components: passive diffusion through the phospholipid bilayer of the cell membrane and facilitated transport mediated by specific membrane proteins. Several types of FA transporter proteins have been identified. The most studied include the FA translocase (FAT/CD36), the plasma membrane-bound FA binding protein 1 (FABPpm), and the FA transporter protein family (FATPI) (21–23). Additional components of the FA transport system are the cytosolic fatty acid binding proteins (FABPc), which act as a metabolic sink for FAs after they enter the cell (24).

The recently generated CD36-deficient mouse (25) and the transgenic mouse with muscle CD36 overexpression (26) have documented the important role of CD36 in facilitating FA uptake in vivo. Alterations in CD36 expression had significant impact on insulin responsiveness and diet-induced insulin resistance. The CD36 null mouse, when maintained on a high-starch low-fat diet, exhibits enhanced insulin sensitivity. However, when fed high fructose, this mouse develops hyperinsulinemia and insulin resistance (27), providing a rodent model of human insulin resistance syndrome, also termed syndrome X (26,28).

FA metabolism plays an important role in modulating the functional activity of islet cells. Whereas acute treatment of islets with FA enhances insulin secretion, prolonged exposure to high FFA has negative actions including reduced glucose metabolism, decreased insulin release, and a pro-apoptotic effect (29). The mechanisms

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FA, fatty acid; FFA, free FA; FRET, fluorescence resonance energy transfer; IRS, insulin receptor substrate; KRB, Krebs-Ringer-HEPES; SSO, sulfoconjugimidyl-oleate; UTR, untranslated region; VAMP, vesicle-associated membrane protein.

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underlying these actions remain poorly defined. The aim of our study was to investigate whether pancreatic β-cells express the CD36 FFA transporter and to elucidate its role in FA modulation of glucose-dependent insulin secretion.

**RESEARCH DESIGN AND METHODS**

Isolation and culture of human islets. Islet isolation was performed by the Islet Core Laboratory of the Diabetes Branch at the National Institute of Diabetes, Digestive and Kidney Disease (National Institutes of Health, Bethesda, MD) using the method of Ricordi et al. (30). Human islets were cultured using M199 medium (Gibco BRL/Life Technologies, Rockville, MD) supplemented with 10% fetal bovine serum (Gibco BRL/Life Technologies) and 1% penicillin-streptomycin (Gibco BRL/Life Technologies) in a humid chamber of 5% CO2/95% air at 37°C.

**Cell culture of mice.** Cells were cultured in Dulbecco’s modified Eagle’s medium (Gibco BRL/Life Technologies) containing 10% fetal bovine serum, 1% penicillin-streptomycin, and 14 μmol/β-mercaptoethanol (Sigma-Aldrich, St. Louis, MO).

The CHO cell line was stably transected with the pcDNA3 plasmid containing the human CD36 (CHO/CD36) (American Type Culture Collection, Manassas, VA). Transfected cells were cultured using RPMI 1640 medium (Gibco BRL/Life Technologies) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. CHO/CD36 cells were used in this study as a positive control for human CD36.

**RNA preparation and RT-PCR.** Cellular pellets obtained from MIN6 and CHO/CD36 were immediately homogenized in 1 ml TriZol (Gibco BRL/Life Technologies). Total RNA was extracted using a Qiagen extraction kit (Gibco BRL/Life Technologies) containing 25 mmol/l-glucose (Gibco BRL/Life Technologies) supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and 14 μmol/β-mercaptoethanol (Sigma-Aldrich, St. Louis, MO).

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**Identification of CD36 transcripts.** Different CD36 transcripts originating from alternative promoters were amplified by using a common reverse primer placed on the first coding exon (5'-AGT AGC CCA CAG TCG TTC-3') together with transcript-specific forward primers (5'-TTG TGC TTT TCC ATC GGA CTT C-3' for A, 5'-TTG ATT GAA AAA TTC TCT GGA-3' for B, 5'-CC TAC ATC TCC GAA AGC AAG C-3' for C, 5'-CCA AAA GCA AGG AGG CAG C-3' for D, 5'-AGA AGT ATG TAT AGA GGA GGA CAG AAA AG-3' for E).

**Protein extraction and Western blotting.** Cells washed twice with PBS were exposed (5 min, room temperature) to 500 μl M-Per mammalian protein extraction reagent (Pierce, Rockford, IL) containing 50 μl protease inhibitor cocktail (Pierce). The lysate was centrifuged at 3,000 rpm for 15 min at 4°C, and an aliquot of the supernatant was used for protein quantification (Bio-Rad, Hercules, CA). Equal amounts of protein were run on a 7.5% SDS-PAGE gel and transferred to a nitrocellulose membrane (Bio-Rad). To confirm transfer completeness, the membranes were stained with ponceau S (Sigma-Aldrich). The membranes were rinsed with PBS and incubated (overnight at 4°C) with an anti-murine CD36 monoclonal antibody (Santa Cruz Biotechnology; Santa Cruz, CA) diluted 1:100 in PBS-Tween with 3% BSA. Immunodetection was performed by using a horseradish peroxidase–conjugated secondary antibody (Santa Cruz Biotechnology) diluted 1:2,000 in PBS-Tween with 3% BSA. To determine whether the identified protein was glycosylated, the protein extract was digested with either endoglycosidase H or N-glycosidase F enzymes (New England Laboratories, Beverly, MA) overnight at 37°C.

**Immunofluorescence.** Human islets were fixed with 4% paraformaldehyde (Sigma-Aldrich). The slides were incubated, 4°C overnight, with a guinea pig anti-insulin polyclonal antibody (DAKO, Carpertina, CA) (1:80 dilution) and a mouse anti-CD36 monoclonal antibody (Santa Cruz Biotechnology) (1:100 dilution). After several washes in PBS, an anti–guinea pig rhodamine-conjugated secondary antibody (1:40 dilution) and an anti-mouse fluorescein-conjugated secondary antibody (1:100 dilution) were added for 1 h at room temperature.

MIN6 cells were subjected to immunofluorescence studies for CD36 and insulin, as described for human islets. In addition, cells were stained with a goat polyclonal anti-GLUT2 antibody (Santa Cruz Biotechnology) (1:200 dilution), followed by incubation with a mouse anti-goat rhodamine-conjugated secondary antibody (1:500 dilution). All secondary antibodies were purchased from (Clontech Laboratories, Palo Alto, CA).

Fluorescence resonance energy transfer (FRET) analysis was performed (31) using a laser confocal microscope Leica TCSPP (Leica Microsystems Heidelberg, Mannheim, Germany). Computer-generated photo-bleaching was used to assess the degree of proximity between insulin and CD36 in the cytoplasm of β-cells.

**Subcellular fractionation.** Cell fractionation was used to examine possible association of CD36 with insulin secretory vesicles. Sucrose gradients fractionation was performed according to Zingg et al. (32). Briefly, cells from confluent 10-cm plates were harvested in 2 ml buffer (200 mmol/l sucrose, 50 mmol/l NaCl, 2 mmol/l EDTA, 10 mmol/l HEPEs-NaOH, pH 7.2, 1 mmol/l phenylmethylsulfonyl fluoride) and homogenized manually with 10 passages through a 23-G needle. The homogenate was centrifuged (1,770g for 6 min at 4°C), and the resulting postnuclear supernatant (1.2 ml) was applied to the top of 1.5-mL sucrose gradient steps (0.2, 0.4, 0.6, 0.8, 1.0, 1.4, and 1.8 mol/l sucrose in 10 mmol/l HEPEs-NaOH, pH 7.2, and 2 mmol/l EDTA).

**Fluorescent microscopy.** At 4°C, cells were centrifuged at 55,000 g for 20 min in a Beckman SW 41 Ti rotor (Beckman, Fullerton, CA). 1-mL fractions were collected from the top to the bottom, precipitated with 15% trichloroacetic acid, and subjected to Western blotting analysis. The pellet for each fraction was dissolved in 50 μl SDS sample loading buffer and then separated on 10% SDS polyacrylamide gel and transferred to a pure nitrocellulose membrane (Bio-Rad). Membranes were probed with antibodies recognizing insulin (1:250 dilution), vesicle-associated membrane protein (VAMP)-2 (1:250 dilution) (Santa Cruz Biotechnology) and CD36 (1:100 dilution), and insulin receptor substrate (IRS)-1 (1:200 dilution) (Santa Cruz Biotechnology). Immunodetection was performed by using horse radish peroxidase–conjugated antibodies (1:2,000 dilution) (Santa Cruz Biotechnology).

**Palmitate uptake.** MIN6 cells, cultured for 24 h on a 6-well plate, were washed twice with 1 ml Krebs-Ringer-HEPES (KRH) buffer (29 mmol/l NaCl, 5 mmol/l NaHCO3, 4.8 mmol/l KCl, 1.2 mmol/l KH2PO4, 1 mmol/l CaCl2, 1.2 mmol/l MgCl2, 2.5 mmol/l glucose, and 10 mmol/l HEPES, pH 7.4) containing 0.2% FA-10% BSA (Sigma-Aldrich). The transport solution contained 0.1 mmol/l palmitate complexed to BSA at a ratio of 0.75, in KRH buffer and 4 × 106/cm2 ml 9.0-10-7 M NaI (v/v 0% sodium eq). This was followed by medium aspiration, and the dish was washed twice with 1 ml cold stop solution (ice-cold KRH buffer with 200 mmol/l dgluconate). The cells were then lifted off the dish with 1 ml trypsin (Gibco BRL/Life Technologies) and centrifuged to yield a pellet that was counted for radioactivity (Beckman, Palo Alto, CA). Primers were identical to those used for RT-PCR. An aliquot of the RT-PCR product was digested with BanII and PstI (Gibco BRL/Life Technologies) at 37°C. The digestion products were analyzed by DNA electrophoresis on 1% agarose and 2% NuSieve gels (Cambrex Bio Science Rockland, Rockland, ME).
Insulin secretion of MIN6 cells treated with palmitate and SSO. MIN6 cells, cultured in 12-well plates until 80% confluent, were switched to Dulbecco's modified Eagle's medium containing 6 mmol/l glucose for 4 h. Acute (1 h) and long-term (24 h) incubations with palmitate and SSO were then performed. In acute experiments, cells were exposed to palmitate (0.5 mmol/l) and SSO (0.5 or 0.05 mmol/l, diluted in medium with 0.05% DMSO and 0.5% BSA) or vehicle (0.05% DMSO/H2O/0.5% BSA, diluted in medium) in the presence of 6 or 15 mmol/l glucose. In long-term experiments, cells were exposed to SSO or vehicle in the presence of 6 mmol/l glucose. At the end of the incubations, the medium was removed, and the dishes were washed twice with PBS. The cells were then challenged with 15 mmol/l glucose for 60 min at 37°C, or re-exposed to fresh medium with 6 mmol/l glucose. The medium and cell pellet were collected. The medium was analyzed for insulin (rat insulin RIA kit; Linco Research, St. Charles, MO) while the cell pellet was washed twice with PBS and exposed (5 min at room temperature) to 500 μl M-Per mammalian protein extraction reagent containing 50 μl protease inhibitor cocktail (both from Pierce, Rockford, IL). The cell lysate was centrifuged (3,000 rpm, 15 min, 4°C), and an aliquot of the supernatant was used for protein quantification (Bradford Protein Assay; Bio-Rad). Protein content was used to normalize for insulin release into the medium.

Fluorescence-activated cell sorter analysis to assess cell viability. Percentage of living, necrotic, and apoptotic cells was evaluated by flow cytometry (FACS; Becton Dickinson, San Jose, CA) using the Annexin-V-Flous Staining kit. The necrotic cells were identified by their capability of taking up propidium iodide and to stain orange/green; apoptotic cells were recognized by the Annexin-V and stained green only, whereas living cells were not stained. Briefly, MIN6 cells were cultured for 24 h in the presence of 0.5 mmol/l palmitate, 0.5 mmol/l SSO, or vehicle alone. The cells were then collected from the culture flasks and washed twice with PBS and then centrifuged at 200 g for 5 min. The cell pellet was suspended in 100 μl staining solution and incubated for 15 min at room temperature. Flow cytometry analysis was performed with a FACScan cytometer (Becton Dickinson, Franklin Lakes, NJ) using the LYSIS II analyzer program.

Statistical analysis. The data were expressed as means ± SE. Overall group mean differences were assessed by ANOVA. The homogeneity of variance assumption was checked using Levene's test. When the ANOVA indicated significant differences, Tukey post hoc tests were used to identify significant differences between individual treatment groups. Planned comparisons were performed by one-degree-of-freedom contrasts using the ANOVA mean square error as the best estimate of the population variance. Planned comparisons were used to investigate whether treatment with palmitate altered the secretion of insulin and to demonstrate that inhibition of CD36-mediated transport of palmitate had a significant capability of reversing the effect of palmitate itself. Calculations were performed using SAS version 8.2 (SAS Institute, Cary, NC).

RESULTS

RT-PCR and DNA sequencing for CD36. Human pancreas and MIN6 cell cDNA was used to amplify the CD36 mRNA by PCR. Human muscle, mammary gland, and CHO-CD36 cDNA were used as positive controls.

As predicted on the basis of the primers location, a fragment of 428 bp was obtained (Fig. 1). To confirm the identity of the amplified fragment, we subjected it to restriction enzyme digestion and micro-sequencing analysis. Two enzymes were used: BamHI and PsI. BamHI digested the PCR product at position 549 of the known human CD36 sequence, resulting in two fragments of 244 and 183 bp; both were identified by gel electrophoresis (Fig. 1). PsI is known to digest CD36 in three positions, producing a 42-, 108-, and 277-bp band. Although the 42-bp band was not detectable, likely because of its very small size, the 108- and 277-bp bands were clearly apparent (Fig. 1). Micro-sequencing analysis further confirmed that the amplified RT-PCR product shared a 100% homology with the known nucleotide sequence of the human CD36 (data...

To further characterize the pattern of CD36 expression, we tested islet RNA for the presence of the three alternative transcripts that have been described in the literature as the result of alternative promoters (32,33). These cDNA forms (A, B, and E in Fig. 2) share the same coding region but have different 5’ untranslated regions (UTRs). All three transcripts could be detected in islets at levels similar to those in skeletal muscle. Alignment of CD36 mRNAs from public databases with the genomic sequence revealed the existence of two additional previously unrecognized transcripts carrying an alternative first exon (transcript C in Fig. 2) or additional 5’ UTR exons (transcript D in Fig. 2). Remarkably, the start site of one of these transcripts (form D) is placed ~200 Kb 5’ of the start of all other ones (Fig. 2A). Both transcripts were expressed in islets and skeletal muscle (Fig. 2B). Transcript C appeared to be abundant in both tissues, whereas form D appeared to be preferentially expressed in islets.

**CD36 Western blotting and de-glycosylation.** Western blot analysis of protein extracts from human islets and MIN6 cells revealed the presence of an ~70-kDa band recognized by the anti-human CD36 and anti-mouse CD36 antibody, respectively. To investigate the presence of carbohydrates and the biochemical nature of the carbohydrate molecules in the CD36 of human islets and MIN6 cells, aliquots of protein extract were subjected to a deglycosylation digestion followed by Western blot analysis. Using N-glycosidase F and endoglycosidase H, we confirmed the presence of glycosylation sites and obtained information regarding the type of carbohydrates linked to CD36 (Fig. 3). CD36, expressed in human islets and MIN6 cells, was digested only by N-glycosidase F and not by endoglycosidase H, suggesting that the expression of CD36 by insulin-secreting cells leads to the production of a glycoprotein containing common classes of N-glycan chains, which are typically cleaved by N-glycosidase F. Treatment with endoglycosidase H showed no apparent digestion of carbohydrates, suggesting that CD36 does not contain high mannose and hybrid type asparagine-linked glycans. The data indicate that pancreatic β-cells express a mature CD36 protein that is highly glycosylated.

**Insulin/CD36 double immunofluorescence in human islets and MIN6.** To help localize CD36 in human islets, we performed double immunostaining for the detection of CD36 and insulin (Fig. 4A and B). The merge of fluorescence for CD36 and insulin (Fig. 4C) indicated a high degree of colocalization. Similar data were obtained in MIN6 cells (Fig. 4D–F), where colocalization of CD36 and insulin could also be documented.
quenching for one of the two proteins detected, this reflects a close molecular association between the two. FRET analysis for insulin and CD36 in MIN6 cells supported colocalization of the two proteins, since fluorescence for CD36 increased after photo-bleaching the signal for insulin (Fig. 5A–F). In contrast, FRET analysis for CD36 and GLUT2 demonstrated no change in fluorescence emission after photo-bleaching the signal for GLUT2 (Fig. 5G–L).

Figure 6 shows the FRET efficiency of control (Fig. 6A—C) and CD36 overexpressing cells (Fig. 6D—F). The FRET efficiency was calculated using the following equation:

\[ \text{FRET efficiency} = \frac{I_{	ext{excited}} - I_{	ext{bleached}}}{I_{	ext{excited}}} \]

where \( I_{	ext{excited}} \) is the fluorescence intensity before bleaching and \( I_{	ext{bleached}} \) is the fluorescence intensity after bleaching. The FRET efficiency was found to be higher in CD36 overexpressing cells compared to control cells, indicating a stronger interaction between the two proteins.
and B) and palmitate-treated (Fig. 6C and D) MIN6 cells, stained for insulin (red) and CD36 (green). After dequenching, the FRET efficiency was evaluated as equal to 50.6% in control cultures and 54.5% in cells cultured in the presence of palmitate, indicating that palmitate does not alter the degree of colocalization between insulin and CD36.

**Subcellular fractionation.** To further confirm the intracellular association of CD36 and insulin, MIN6 cells were fractionated by ultracentrifugation using a discontinuous sucrose density gradient. β-Cells contain two types of secretory vesicles: insulin-containing vesicles and synaptic-like microvesicles, which contain γ-aminobutyric acid (34). Insulin-containing vesicles express a protein termed VAMP-2, which has been characteristically used to identify vesicle-associated proteins. Figure 7 shows immunoblots of the various cell fractions probed with antibodies recognizing CD36, insulin, VAMP-2, and IRS-1. The majority of immunoreactivity for insulin and CD36 was detected in fraction 10, where VAMP-2 was also detected. CD36 was also present in fraction 12 (plasma membrane fraction). Approximately 65–70% of overall CD36 immunoreactivity was associated with the VAMP+ fraction while 10–15% was associated with the cell membrane.

Immunoreactivity for IRS-1 was used as a control, since it is a non–granule-associated protein. As shown in Fig. 7, IRS-1 showed a very different pattern of subcellular distribution and was recovered primarily in fractions associated with cytosolic proteins and most abundantly in fraction 5.

**Effect of the CD36 inhibitor SSO on palmitate uptake by MIN6 cells.** Because a fraction of cellular CD36 was recovered in the plasma membrane fraction, we examined whether it is directly involved in FFA uptake by β-cells. The effect of the specific and irreversible CD36 inhibitor SSO on palmitate uptake was examined in MIN6 cells. Treatment of MIN6 cells with 0.5 mmol/l SSO was associated with 46.9.7% inhibition of palmitate uptake compared with cells treated with vehicle alone (P < 0.01).

**Effect of SSO on insulin secretion.** To examine whether CD36 function affects glucose-sensitive insulin secretion by β-cells, we compared effects of palmitate on insulin release in the absence or presence of the CD36 inhibitor SSO. A 1-h exposure to palmitate was associated with an enhancement of insulin secretion, in line with previous reports (35). Results were similar whether the medium contained 6 mmol/l (P < 0.01) or 15 mmol/l glucose (P < 0.01) (Fig. 8).

Inclusion of SSO in the medium abolished the palmitate effect (P < 0.01 for cells cultured in the presence of 0.5 mmol/l SSO, with either 6 or 15 mmol/l glucose).

Long-term (24-h) exposure of MIN6 cells to palmitate produced an inhibition of glucose-dependent insulin secretion (P < 0.001; for cultures maintained in either 6 or 15 mmol/l glucose), similarly to the previously described...
reduction of insulin secretion from isolated islets chronically exposed to FFAs (36). Inclusion of the CD36 inhibitor SSO in the culture medium reversed the inhibitory effect of palmitate on insulin secretion, demonstrating the specificity of the effect ($P < 0.001$ for cultures maintained in either 6 or 15 mmol/l glucose) (Fig. 8).

Fluorescence-activated cell sorter analysis to assess cell viability. Fluorescence-activated cell sorter analysis, conducted to obtain a quantitative analysis of the effect of a 24-h exposure to either 0.5 mmol/l palmitate or 0.5 mmol/l SSO, or vehicle alone on the viability of MIN6 cells, did not demonstrate any significant difference among the different treatment groups (Table 1).

DISCUSSION

In the present study, we demonstrated that human pancreatic islet cells express the CD36 protein, which was previously shown to be an important facilitator of FA uptake by adipose (25) and muscle (37) cells. RT-PCR, DNA sequencing, and restriction enzyme digestion proved that the identified mRNA was identical to that previously characterized in muscle and fat cells. As in these tissues, the protein is heavily glycosylated with N-linked carbohydrates constituting $\sim 30\%$ of its apparent molecular weight. Islet CD36, like that of muscle and fat cells, appears to function in FA uptake, as demonstrated by the studies using the specific inhibitor SSO. CD36 may play an important role in modulating islet function through regulating FA utilization by islets. FAs are an important energy source but also function as signaling molecules to modulate cellular pathways including insulin secretion by islets. FAs are thought to contribute about one-third of the released insulin under physiological conditions (1). Acutely, FAs enhance glucose-induced insulin secretion and appear essential for maintaining it during fasting (38). These acute effects of FAs on modulate insulin secretion could be mediated by one or more mechanisms, including providing ATP, altering glucose metabolism, modifying ionic channels, or affecting the efficiency of granule exocytosis (39). The effect of FAs on granule exocytosis (40) is especially intriguing, since we have documented by double immunostaining and FRET that most of CD36

**FIG. 6.** Effect of palmitate on protein colocalization: quantitative analysis of FRET efficiency. After immunostaining for insulin (red) and CD36 (green), MIN6 cells treated with palmitate or vehicle were subjected to quantitative analysis for protein colocalization. The red square indicates the analyzed area. A: Untreated MIN6 cells stained for insulin and CD36 after dequenching. B: Calculated FRET efficiency of analyzed area in A. C: MIN6 cells treated with 10 mmol/l palmitate stained for insulin and CD36 after dequenching. D: Calculated FRET efficiency of analyzed area in C. Quantitative analysis of protein colocalization is shown to the right of panels B and D. FRET analysis was repeated twice, and the data obtained provided similar results.

**FIG. 7.** Subcellular localization of CD36 and insulin. Postnuclear supernatants from MIN6 cells were separated on a sucrose step gradient and analyzed by Western blotting using CD36 antibody and insulin antibody and anti-VAMP-2 and IRS-1. This experiment was repeated twice, providing results very similar to the one depicted here. The numerical values of density, shown at the top of the figure, are calculated as fractions of the density of a 0.2-mol/l solution of sucrose dissolved in water.
colocalizes with insulin. This finding was further supported by CD36 migration with the vesicular protein VAMP-2 in subcellular fractions. Thus, the possibility that FA binding to CD36 on granules could facilitate granule docking on the plasma membrane should be investigated in future studies. In this context, it is interesting to note that CD36 has been localized on α-granules as well as on plasma membranes of platelets and has been hypothesized to play a role in surface expression of α-granules during platelet activation (41).

In contrast to acute exposure, prolonged treatment (24 h) of islets with FAs inhibited insulin secretion. This is in line with previous reports that documented negative effects of long-term exposure of β-cells to high FAs on intracellular insulin levels and glucose-dependent insulin secretion (29,42). Other changes noted were an increase in intracellular lipid content and a significant reduction of glucose oxidation and utilization. The combination of FAs and high glucose was also shown to damage islet viability, since the number of apoptotic cells increased (42).

Inhibition of insulin secretion by palmitate, like its acute stimulatory effect, was prevented by inclusion of the CD36 inhibitor SSO. This finding indicates that high levels of CD36-facilitated FA uptake could play a role in β-cell lipotoxicity, which is thought to contribute to the etiology of type 2 diabetes. In this context, it has been shown that hyperglycemia enhances CD36 expression via a translational mechanism (43), which would further exaggerate lipid uptake and accumulation. Increased CD36 expression has been documented in human type 2 diabetes (44,45). Our findings would suggest that targeting CD36 on islets might be beneficial in chronic diabetes to help prevent lipid accumulation and lipotoxicity.

The regulation of CD36 expression appears to be as complex in islets as in other tissues. It has been previously

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<th>Cells and treatment</th>
<th>Necrosis (%)</th>
<th>Apoptosis (%)</th>
<th>Living (%)</th>
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<tr>
<td>MIN6</td>
<td>7.0 ± 0.68</td>
<td>9.7 ± 2.7</td>
<td>83.3 ± 4.3 (NS)</td>
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<td>MIN6 + 0.5 mmol/l palmitate</td>
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<td>12.2 ± 2.7</td>
<td>76.1 ± 5.2 (NS)</td>
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<td>MIN6 + 0.5 mmol/l SSO</td>
<td>8.5 ± 1.1</td>
<td>9.7 ± 1.2</td>
<td>81.8 ± 4.2 (NS)</td>
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Data are means ± SE of four independent experiments. Statistical significance was determined by ANOVA.
reported that glucose regulates CD36 by a mechanism involving ribosomal re-initiation (43). Expression is also under transcriptional regulation by the peroxisome proliferator-activated receptors in a tissue-specific manner (33).

We detected a total of five alternative transcripts differing in their 5’t UTRs. Three of these forms have been previously described in the literature as originating from alternative promoters having different sensitivity to transcriptional regulators such as peroxisome proliferator-activated receptors (33). The other two are newly identified transcripts that appear to be under the control of independent promoters, one of which is placed >200 Kb from the others. The occurrence of multiple promoters and 5’t UTRs would be predicted to allow the control of gene transcription by diverse signals in a cell type–specific manner. In this regard, it is interesting to note that one of the new transcripts—that from the most distal promoter—seems to be especially abundant in islets. Whether this truly reflects an islet-specific regulation of this promoter will have to be determined by quantitative assays aimed at determining the relative abundance of the five transcripts in different tissues and the transcriptional regulation of the different promoters.

In summary, the importance of FAs in maintaining or modulating the function and viability of insulin-producing cells has long been recognized. In the present study, we provide a potential mechanism by which ambient FAs may modulate the functional activity of insulin-producing cells by documenting that these cells express the FA-translocon CD36 and that CD36 function can significantly affect insulin secretion. Targeting the CD36 receptor may provide a novel approach to selectively modify islet FA metabolism and its effects on insulin secretion and cell viability.

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