Thiazolidinediones upregulate impaired fatty acid uptake in skeletal muscle of type 2 diabetic subjects


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Although long-chain free fatty acids (FFA) serve as a major fuel for skeletal muscle at rest and with moder-

ate activity (59), the identity of the mechanisms by which FFA are transported across the membrane re-

mains unclear. FFA may diffuse passively across the cell membrane, depending on the rate of cellular me-

tabolism (29). In addition, it is suggested that a portion of transmembrane flux of FFA is protein mediated (1).

In support of this view, it has been shown that FFA uptake in adipose tissue (4), heart (63), and skeletal

muscle (67) exhibits saturability, specificity, and sen-

sitivity to protein modifiers.

One possible mediator of FFA transport is fatty acid translocase (FAT). FAT has been shown to be identical
to CD36, the scavenger receptor for oxidized LDL (48).
FAT/CD36 is abundantly expressed in tissues with high metabolic capacity for fatty acids (2, 39). FAT/CD36 expres-
sion correlates well with FFA transport into rat heart as well as red and white muscle vessels (2, 39). Transgenic mice overexpressing FAT/CD36 in muscle show an enhanced ability of muscle to oxidize FFA in response to stimulation and contraction, together with decreased triglyceride and FFA levels (31). FAT/CD36 deficiency has been proposed as a possible mechanism responsible for insulin resistance, defective fatty acid metabolism, and hypertriglyceridemia in the spontaneously hypertensive rat (56). Another proposed mediator of FFA uptake is plasma membrane fatty acid-binding protein (FABPpm). FABPpm expression correlates with FFA uptake or utilization in a variety of different circumstances (6, 68). Expression of FABPpm in 3T3 fibroblasts is associated with an increase in FFA uptake rates, and this increase reflects the addition of a saturable, high-affinity component inhibited by antibodies against FABPpm (70). A third possible mediator of FFA uptake is fatty acid transport protein (FATP-1). FATP-1 is expressed in tissues active in fatty acid utilization like skeletal muscle, heart, and fat (7). Overexpression of FATP-1 in cells is asso-
ciated with an increase in uptake of FFA (61).

Because plasma FFA levels are increased (36, 64) and fatty acid utilization is impaired in type 2 diabetic patients (36), regulation of FFA uptake may also be impaired in this situation. Insulin decreases FFA up-
take in muscle in vivo (69), possibly by an indirect

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effect to decrease FFA plasma levels, through inhibition of lipolysis in adipocytes (35). There is some evidence that insulin can activate FFA uptake into several cell types (20, 48), possibly by stimulating FAT/CD36 translocation to the plasma membrane (37). It is unclear whether insulin effects on FFA uptake into muscle represent another process in which insulin action is impaired in type 2 diabetic patients.

Troglitazone (Tgz), rosiglitazone (Rgz), and pioglitazone (Pio) are members of the thiazolidinedione class of insulin-sensitizing drugs (47). They have been shown to lower plasma FFA levels in type 2 diabetic patients (41, 53). Thiazolidinediones have been identified as peroxisome proliferator-activated receptor-γ (PPARγ) activators (62). Both FAT and FATP have been shown to be regulated by PPARγ (42, 46) and may participate in a mechanism by which Tgz and Rgz increase FFA uptake in vitro into cultured adipocytes (43).

The aim of the present study was to study the regulation of FFA transport by acute insulin and chronic thiazolidinedione treatment and compare these effects in cultured skeletal muscle cells of type 2 diabetic and nondiabetic subjects. This system has proved useful for muscle cells cultured from type 2 diabetic subjects, which have been shown to retain defects in insulin action and glucose metabolism reflective of those seen in vivo (18, 30). To gain insight into underlying mechanisms for regulation of FFA uptake, the effects of thiazolidinediones on FAT/CD36, FATP, and FABPpm mRNA and/or protein expression were also studied, along with the ultimate endpoint for FFA uptake, FFA oxidation.

MATERIALS AND METHODS

Human subjects. A total of 30 nondiabetic and type 2 diabetic subjects provided muscle tissue for these studies. All subjects had a 2-h 75-g oral glucose tolerance test. Normal diabetic subjects provided muscle tissue for these studies. All subjects did not exercise for the procedures were performed after an overnight fast. None of the subjects was involved in a vigorous exercise program. Procedures were performed before insulin infusion. The biopsy and clamp (GDR) was determined during the last 30 min of the clamp (66). Needle biopsies of the vastus lateralis muscle were performed before insulin infusion. The biopsy and clamp procedures were performed after an overnight fast. None of the subjects was involved in a vigorous exercise program. Subjects did not exercise for ≥24 h before the procedure. The clinical characteristics of these subjects are summarized in Table 1. The diabetic subjects were significantly older and more obese than the nondiabetic individuals. None of the diabetic subjects had any comorbidity that would influence glucose or lipid metabolism. The diabetic subjects were treated in the following manner: insulin, n = 1; glyburide, n = 8; metformin, n = 1; glyburide plus metformin, n = 3; no therapy, n = 2. Insulin resistance in the diabetic subjects was confirmed by the reduced GDR (Table 1). Although fasting FFA levels did not differ between groups, FFA levels at the completion of the insulin clamp were higher in the diabetic subjects (0.096 ± 0.016 vs. 0.045 ± 0.008 mM in nondiabetic, P < 0.005), indicating an impaired ability of insulin to suppress lipolysis. The experimental protocol was approved by the Committee on Human Investigation of the University of California, San Diego. After explanation of the protocol, written informed consent was obtained from all subjects.

Materials. Cell culture materials were purchased from Irvine Scientific (Irvine, CA) except for skeletal muscle basal medium, which was obtained from Clonetics (San Diego, CA). Human biosynthetic insulin was kindly supplied by Eli Lilly (Indianapolis, IN). Bovine serum albumin (BSA; Cohn fraction V) was purchased from Boehringer Mannheim (Indianapolis, IN) and phloretin from ICN Biomedicals (Costa Mesa, CA). [U-14C]palmitate, [9,10-2H]palmitate, l-[U-14C]-glucose, and [1-14]Hdeoxyglucose were obtained from New England Nuclear Life Science Products (Boston, MA). Unlabeled palmitate, FFA-free BSA, deoxyglucose, and l-glucose were purchased from Sigma (St. Louis, MO). TRizol reagent was purchased from Gibco-BRL (Gaithersburg, MD), Nyttran plus membrane from Schleicher & Schuell (Keene, NH), DECAprime II Random Priming Kit and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA from Ambion (Austin, TX), Chroma spin columns from Clontech (Palo Alto, CA), QuickHyb from Stratagene (San Diego, CA), and BiMax film from Eastman Kodak (Rochester, NY). FAT/CD36, FATP-1, and FABPpm cDNAs were kindly provided by Drs. Azeddine Ibrahim of State University of New York (Stony Brook, NY), Andreas Stahl of the Whitehead Institute (Cambridge, MA), and Joseph Mattingly of the University of Missouri (Kansas City, MO), respectively. Reagents for electrophoresis and the AG-1X8 resin were obtained from Bio-Rad (Richmond, CA). Monoclonal CD36 and actin antibodies raised in mice were purchased from Serotec (Raleigh, NC) and Sigma, respectively. Anti-mouse IgG complexed to horseradish peroxidase and Hyperfilm were from Amersham (Arlington Heights, IL). SuperBlock and SuperSignal Chemiluminescent Substrate Kit were obtained from Pierce (Rockford, IL). Tgz was a kind gift of Dr. Alan Saltiel (Pfizer Parke-Davis Pharmaceuticals, Ann Arbor, MI); Rgz was provided by Dr. Stephen Smith (GlaxoSmithKline, Harlow, UK); and Pio was supplied by Dr. A. Kozai (Takeda Pharmaceuticals America, Lincolnshire, IL).

Human skeletal muscle cell culture. The methods for muscle biopsy of the vastus lateralis and cell isolation and growth have been described in detail previously (30). At 80–90% confluence, cells were fused for 4 days in α-MEM containing 2% FBS, 1% fungibact, 100 U/ml penicillin, and 100 µg/ml streptomycin. GDR was confirmed by the reduced GDR (Table 1). Although fasting FFA levels did not differ between groups, FFA levels at the completion of the insulin clamp were higher in the diabetic subjects (0.096 ± 0.016 vs. 0.045 ± 0.008 mM in nondiabetic, P < 0.005), indicating an impaired ability of insulin to suppress lipolysis. The experimental protocol was approved by the Committee on Human Investigation of the University of

Table 1. Clinical characteristics of subjects

<table>
<thead>
<tr>
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<th>Nondiabetic (n = 15)</th>
<th>Type 2 Diabetic (n = 15)</th>
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<tbody>
<tr>
<td>Gender (F/M)</td>
<td>(2/13)</td>
<td>(1/14)</td>
</tr>
<tr>
<td>Age</td>
<td>43 ± 3</td>
<td>53 ± 2*</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.4 ± 1.3</td>
<td>34.3 ± 1.7*</td>
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<tr>
<td>Fasting glucose, mM</td>
<td>4.9 ± 0.1</td>
<td>8.6 ± 0.1</td>
</tr>
<tr>
<td>Fasting insulin, pM</td>
<td>79 ± 13</td>
<td>180 ± 34*</td>
</tr>
<tr>
<td>Hb A1c, %</td>
<td>5.6 ± 0.1</td>
<td>7.7 ± 0.7*</td>
</tr>
<tr>
<td>GDR, mg·kg⁻¹·min⁻¹</td>
<td>11.27 ± 0.75</td>
<td>6.25 ± 0.61*</td>
</tr>
<tr>
<td>FFA, mM</td>
<td>0.404 ± 0.049</td>
<td>0.542 ± 0.088</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>117 ± 17</td>
<td>161 ± 33</td>
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Results are averages ± SE. GDR, glucose disposal rate; FFA, free fatty acids; TG, triglycerides. *P < 0.05 compared with nondiabetic subjects.
of the thiazolidinediones were determined in preliminary studies to provide maximal stimulation of palmitate oxidation and FFA uptake. Agents were dissolved in DMSO to a final concentration of 0.05%. Previous work has shown that this treatment protocol does not alter the extent of differentiation of myocytes into mature myotubes (52).

FFA uptake assay. FFA transport was measured on cells grown on 12-well plates. After completion of differentiation and treatment, the cells were rinsed twice with serum-free α-MEM containing 0.1% BSA, pH 7.4. Cells were then incubated in this medium at 37°C with 0 or 30 nM phloretin. After 70 min, the medium was removed by rinsing the cells four times with room temperature reaction buffer (in mM: 150 NaCl, 5 KCl, 1.2 MgSO4, 1.2 CaCl2, 2.5 NaH2PO4, and 10 HEPES and 0.1% BSA, pH 7.4). Uptake was initiated by replacing the reaction buffer with 1 ml of reaction buffer premixed with substrate (0.3 μCi [14C]palmitate, final concentration 20 μM). The substrate was prepared under N2 by mixing with FFA-free BSA (12); concentration of the stock solution was confirmed by colormetric assay for FFA (Wako Chemicals, Richmond, VA). Transport was stopped after 1 min by aspirating the substrate and washing five times with 4°C PBS. The cells were solubilized by incubating them in 0.5 ml of 0.1 N NaOH with shaking. Protein content was determined on an aliquot of the cell suspension by the Bradford method (11). The remaining volume was transferred to a scintillation vial, and scintillation fluid was added and radioactivity measured. Total counts were determined by placing 0.5 ml of substrate containing reaction buffer directly into the scintillation vial. To compensate for FFA adhesion to the well and cells, zero-time counts were subtracted. Transport was normalized to the amount of cell protein. All measurements were performed in triplicate. The intra-assay coefficient of variation was 7%. Whereas phloretin has been shown to inhibit a number of carrier-mediated processes (4), for this report protein-mediated uptake is operationally defined as the difference between total uptake and that measured in the presence of phloretin for each individual set of cells. This correction would account for the radioactivity that entered cells by diffusion and FFA adsorbed to the cell surface that was not removed during the washing procedure.

Glucose uptake assay. Glucose uptake measurements were carried out as described previously (30). Medium was added to the cells together with insulin (0 or 33 nM), and the cells were incubated for 60–90 min in a 95% O2/5% CO2 incubator before washing and transport assay. An aliquot of the suspension was removed for protein analysis using the Bradford method. Uptake of 1-glucose was used to correct each sample for the contribution of diffusion.

Measurement of palmitate oxidation. The procedure for assaying palmitate oxidation is a modification (10) of a method established for adherent cells (58). Cells were incubated in serum-free medium containing 10 μl of substrate (0.2 μCi [9,10-3H]palmitic acid, final concentration 2 μM) in a 95% O2/5% CO2 incubator at 37°C for 3 h. After incubation, an aliquot (100 μl) of the culture medium was placed over an ion exchange resin, and the column was washed twice with 0.75 ml of water. Intact palmitate (charged state) was retained by the resin, whereas the oxidized portion of palmitate passed freely through the resin column in the form of water (9,10).

Northern analysis of FAT/CD36, FATP, and FABPpm. Total cellular RNA was isolated from muscle cell cultures with TRIzol according to the manufacturer’s instructions. Ten micrograms of total RNA were separated according to size by electrophoresis through a denaturing formaldehyde 1–1.5% agarose gel and transferred to a Nytran plus membrane. DNA probes for Northern analysis were labeled by the decamer-priming method using the DECaprime kit (Ambion) and purified with Chromaspin columns. Hybridization with FAT/CD36, FATP, and FABPpm, and GAPDH cDNA probes labeled with [α-32P]deoxyctydine triphosphate was carried out at 68°C in 5 ml of QuickHyb, according to the manufacturer’s instructions. To remove nonspecific binding, membranes were washed twice at room temperature with 2× standard sodium citrate (SSC), 0.1% SDS buffer, and then once with 0.2× SSC and 0.1% SDS buffer, followed by one 30-min wash at 60°C with 0.1× SSC and 0.1% SDS buffer. After being washed, membranes were exposed to BioMax film at –70°C. The relative intensities of transcript signals were compared quantitatively with a computer imaging program (NIH Image) by using the GAPDH signal to normalize for loading differences.

Analysis of FAT/CD36 protein expression. Cell protein lysates were prepared as described previously (32). Western blot analysis was done as detailed previously (30). In summary, cell extracts were solubilized in Laemmli buffer, and 10–20 μg of protein were separated according to size on 10% SDS-PAGE gels and electrophoretically transferred to a nitrocellulose membrane. The membrane was blocked for 1–2 h at room temperature with TBS, 0.05% Tween, and 5% nonfat milk, pH 7.4. The monoclonal CD36 antibody was diluted 1:250 in SuperBlock, pH 7.4, and incubated overnight at 4°C. For actin, the membrane was blocked with TBS, 0.05% Tween, and 5% nonfat milk, pH 7.4, overnight at 4°C. The monoclonal actin antibody was diluted 1:2,000 in TBS, 0.05% Tween, and 5% BSA, pH 7.4, and incubated at room temperature for 1 h. The secondary antibody for both CD36 and actin was anti-mouse IgG conjugated to horseradish peroxidase. Proteins were visualized with the SuperSignal Chemiluminescent Substrate Kit and exposed to Hyperfilm.

Statistical analysis. Statistical significance was evaluated using a two-tailed Student’s t-test for paired or unpaired comparisons, where appropriate. Significance was accepted at the P < 0.05 level. Because of limitations in tissue availability, not all experiments in cell cultures were performed in cells from all individuals. There were no differences in the clinical characteristics of the subsets of subjects whose cells were used in each study.

RESULTS

Determination of protein-mediated portion of FFA uptake in nondiabetic and diabetic muscle. Phloretin, a nonselective inhibitor of protein-mediated transport (4), has previously been used to inhibit FFA transport (3, 40). In the present study, phloretin was employed to distinguish between protein-mediated transport and other means of palmitate becoming associated with the cells, including diffusion. Differentiated human skeletal muscle cell cultures were treated with 300 μM phloretin for 70 min preceding transport. Palmitate uptake was measured over 1 min, as uptake was still linear in this time period (data not shown). Phloretin inhibited total uptake by 23–25% (Fig. 1, P < 0.0001). The phloretin effect on palmitate uptake was acutely reversible. Total FFA uptake was similar in muscle cells from nondiabetic (481 ± 40 nmol·mg protein–1 min–1, n = 9) and diabetic (520 ± 121 nmol·mg protein–1 min–1, n = 9) subjects (Fig. 1A). However, the phloretin-inhibitable component, taken to repre-
sent carrier-mediated uptake, was reduced in diabetic muscle cells (78 ± 13 nmol·mg protein⁻¹·min⁻¹) compared with nondiabetic (150 ± 17 nmol·mg protein⁻¹·min⁻¹, P < 0.01) muscle (Fig. 1A). Glucose uptake was measured in cells from the same subjects. There was also a tendency for basal glucose uptake to be impaired in the diabetic cells (12.6 ± 4.8 nmol·mg⁻¹·min⁻¹) compared with nondiabetic (19.6 ± 3.8 nmol·mg⁻¹·min⁻¹) muscle; this result is in agreement with our previous findings (18).

Acute regulation of FFA uptake. To determine whether insulin has a direct acute effect on FFA uptake, differentiated cells were treated with 30 nM insulin for 70 min before assay. Acute insulin treatment caused a modest (+16 ± 5%, n = 5) but significant (P < 0.025) stimulation of the carrier-mediated palmitate uptake (the phloretin-inhibitable portion) into nondiabetic muscle (Fig. 1B). There was no significant insulin effect on FFA uptake in diabetic muscle cells (Fig. 1B); this was true for either absolute values or relative (+19 ± 19%, n = 8, P = not significant) changes. In contrast, muscle cells from these same diabetic subjects retained significant insulin stimulation of glucose transport (+23 ± 10%, n = 5, P < 0.05), although this value was also reduced compared with the insulin effect in nondiabetic muscle (+44 ± 8%, n = 7, P < 0.005).

Chronic regulation of FFA uptake: effects of thiazolidinedione treatment. Because treatment with antidiabetic thiazolidinediones decreases FFA levels in vivo (26, 35), their chronic effect on FFA uptake into muscle cells was studied. Cells were treated with Tgz (11 μM), Rgz (10 μM), or Pio (10 μM) for 4 days during differentiation. None of the agents influenced the differentiation of muscle cells, as indicated by the extent of multinucleation or expression of sarcomere-specific α-actin (not shown), similar to previous results (52). From the example presented in Fig. 2A, it can be seen that Rgz treatment increased total palmitate uptake in nondiabetic muscle cells, whereas uptake in the presence of phloretin was unaltered, indicating that thiazolidinedione treatment increased only the protein-mediated component of uptake. Treatment effects on this carrier-mediated component of FFA uptake are presented in Fig. 2B. For nondiabetic muscle cells, both Tgz (+32 ± 4%) and Rgz (+68 ± 26%) caused significant increases; Pio effects were not measured in non-diabetic cells. In diabetic muscle cells, thiazolidinedione treatment had the result of normalizing the impaired uptake activity (Fig. 2B). The thiazolidinediones were equally potent in elevating uptake in diabetic muscle: Tgz, +87 ± 32%; Rgz, +70 ± 15%; and Pio, +98 ± 9%.

One fate of FFA taken up into the muscle cell is oxidation. Under the same circumstances where FFA uptake was upregulated by thiazolidinedione treatment, β-oxidation of palmitate was also increased by all three agents (Fig. 2C) in both nondiabetic and diabetic muscle cells, in confirmation of previous results (15).

Effects of thiazolidinediones on expression of putative mediators of FFA uptake. To identify which gene products might be responsible for the protein-mediated component of FFA uptake and to gain initial insight into possible mechanisms by which thiazolidinediones could increase FFA uptake, the effects of thiazolidinedione treatment on FAT/CD36, FATP, and FABPpm mRNA expression and FAT/CD36 protein expression were determined.

RNA was isolated from cultures treated with 11 μM Tgz for 4 days during differentiation. FAT/CD36, FATP-1, and FABPpm mRNA expression were determined with Northern blotting. To normalize for loading differences, a GAPDH probe was used. A representative blot is shown in Fig. 3A. mRNAs for FAT/CD36, FATP, and FABPpm were all present in skeletal muscle cell cultures. Quantification of the signals, normal-
ized for loading differences, is shown in Fig. 3B. Tgz treatment upregulated FAT/CD36 mRNA by 193 ± 65% (n = 6, P < 0.05). There was no significant change in either FABPpm or FATP mRNA expression.

Protein was isolated from cultures treated with Tgz (11 μM), Rgz (10 μM), or Pio (10 μM) for 4 days, and Western blotting with a FAT/CD36 antibody was performed; results are shown in Fig. 4. All three thiazolidinediones increased FAT/CD36 protein expression: +90 ± 22% over control (P < 0.005) for Tgz, +146 ± 42% (P < 0.01) for Rgz, and +111 ± 37% (P < 0.025) for Pio.

DISCUSSION

FFA serve as a major fuel for skeletal muscle. However, it remains unclear whether FFA cross the cell membrane solely by simple diffusion or whether there is a protein-mediated component of transport present as well. We employed phloretin, a nonselective inhibitor of carrier-mediated membrane transport processes (4), to distinguish between protein-mediated FFA transport and diffusion and found that phloretin inhibited initial rates of total FFA uptake by ~25%. It has been reported that phloretin inhibits FFA transport into multiple cell types to a varying extent: adipocytes by 65% (3), cardiac myocytes by 79% (40), heart giant vesicles by 35–45% (38, 40), and muscle giant vesicles by 50–55% (10). Thus the contribution of the protein-mediated component of FFA uptake appears to depend...
port of insulin resistance for glucose metabolism in type 2 diabetes is intrinsic to skeletal muscle and not solely the result of the hyperglycemic, hyperinsulinemic, and hyperlipidemic environment characteristic of the condition. In the present work, we found that insulin stimulated the protein-mediated component of FFA uptake into muscle cells of nondiabetic subjects, a behavior similar to that seen in vivo (5). Differences were also seen in FFA uptake between nondiabetic and diabetic muscle cells and in diabetes-related impairments in both basal activity and insulin responsiveness. These differences suggest that at least a portion of defective FFA metabolism in type 2 diabetes is not acquired from the metabolic environment. These in vitro results are in agreement with several reports of impaired in vivo skeletal muscle FFA uptake and disposal measured in type 2 diabetic individuals (8, 29). Defects in FFA uptake and disposal have also been reported in subjects with impaired glucose tolerance in the presence of normal glucose, insulin, and lipid levels (44). Thus there is in vivo and in vitro (this report) evidence that impaired FFA uptake into skeletal muscle in type 2 diabetes is independent of the hormonal and metabolic environment. It should be noted that the type 2 diabetic subjects who supplied tissue for these studies were older and more obese than the nondiabetic subjects; the contribution of these differences to the impairment in FFA uptake is uncertain.

The insulin-sensitizing thiazolidinediones decrease plasma FFA levels in vivo (41, 53). Possible mechanisms for this response could include reduced lipolysis and FFA release by adipose tissue, reduced hepatic lipogenesis (reviewed in Ref. 5), or increased in FFA uptake and FFA release by adipose tissue, reduced hepatic lipogenesis (reviewed in Ref. 5), or increased in FFA uptake into muscle. Indeed, the present results indicate that Tgz, Rgz, and Pio can all increase FFA uptake into muscle cells. Tgz and Rgz have been shown to increase FFA transport into 3T3-L1 adipocytes (24, 42) as well, so it is possible that similar mechanisms are functioning in adipocytes and muscle cells.

Reconciling findings of an in vitro effect of thiazolidinediones to stimulate FFA uptake and oxidation with in vivo results is complicated by the ability of thiazolidinediones to reduce circulating FFA levels. Thus a reduction in whole body FFA disposal or oxidation following thiazolidinedione treatment (50) could be due to a mass action effect of lowered substrate delivery to tissues. Conversely, when palmitate is infused to maintain a fixed level, FFA utilization is augmented after thiazolidinedione treatment (12, 26). This latter situation is closer to the in vitro condition, where the palmitate concentration is also fixed.

A reciprocal relationship between glucose and fatty acid metabolism has been established in many circumstances (57), although there are also situations where this relationship does not appear to hold (19). In light of this relationship, how is it possible to reconcile thiazolidinedione-induced increases in both glucose uptake and FFA uptake? One explanation would be if, at least for the limited time represented by these assays, the glucose were being directed toward storage in glycogen while palmitate was being oxidized to meet...
the immediate energy needs of the cell. If thiazolidinedione treatment were to shift the balance between oxidation of fatty acids and glucose oxidation predominantly to fatty acids, the net effect would be to direct glucose to nonoxidative pathways of utilization. That would be consistent with our finding that troglitazone treatment of human muscle cells, besides increasing glucose uptake, increased glycogen synthase activity and glucose incorporation into glycogen (52). This would also be consistent with the results of Miyazaki et al. (45), who reported that the increase in whole body glucose disposal following pioglitazone treatment of diabetic subjects could be accounted for by the increase in nonoxidative glucose metabolism, usually taken to represent glycogen synthesis.

Thiazolidinedione treatment increased only the protein-mediated component of FFA uptake (Fig. 2), suggesting that thiazolidinediones upregulate transporter levels or increase transporter activity, the latter possibly through translocation to the cell surface. Indeed, we did find that thiazolidinediones upregulated FAT/CD36 mRNA and protein expression, whereas troglitazone did not alter FATP-1 and FABPpm mRNA levels. These results would be in agreement with other reports where PPARγ ligands, including thiazolidinediones, have been shown to increase FAT/CD36 expression in multiple cell types, including adipose tissue and macrophages (23). Other evidence suggests that changes in FATP-1 expression may not play a major role in impairments in FFA uptake in obesity and type 2 diabetes (10). However, there are data that thiazolidinedione treatment increases both FAT and FATP mRNA levels in adipose tissue (42, 46). Thus upregulation of FFA uptake by thiazolidinediones and the specific proteins involved may show tissue and/or species specificity.

From the current results we conclude that thiazolidinediones increase FFA uptake into muscle cells, possibly through upregulation of FAT/CD36 expression. Although FFA uptake into muscle would have a positive impact on circulating FFA levels, lipid accumulation in muscle has repeatedly been shown to be associated with insulin resistance (34, 51, 54, 55) in sedentary individuals. How is this fact reconcilable with the demonstrated ability of thiazolidinediones to improve insulin action? A similar dichotomy is observed for FFA flux in macrophages. In those cells, thiazolidinediones upregulate FAT/CD36 expression and oxidized LDL entry, generating additional ligands for PPARγ, further increasing FAT/CD36 expression and lipid uptake (17, 49). Such a cycle could be considered proatherogenic. However, in macrophages, thiazolidinediones also increase expression of ATP Binding Cassette-A1, the transporter responsible for reverse cholesterol efflux (17); thus cholesterol esters do not accumulate within the cell. A similar balancing act may occur in skeletal muscle cells, as we have shown that thiazolidinediones increase both palmitate uptake and oxidation (Fig. 2), resulting in an increase in overall FFA disposal. Studies indicate that Tgzs acts to increase the mitochondrial component of palmitate oxidation (15). We therefore hypothesize that thiazolidinediones are involved in directing FFA to the mitochondria by upregulating FFA uptake mediators. Directing FFA to the mitochondria for oxidation may prevent them (either in their free form or as triglycerides) from causing insulin resistance. Consistent with this postulate, thiazolidinediones have been found to increase uptake and oxidation of FFA by muscle in several animal models of insulin resistance (16, 33). Other investigators have found a contrasting result, Tgzs inhibition of palmitate oxidation in isolated soleus muscle (13). The reason for this discrepancy is not apparent but may reflect differences in the experimental systems employed. A similar situation of augmented FFA disposal in skeletal muscle occurs with endurance training, which increases triglyceride localization around the mitochondria (65). Interestingly, trained individuals have elevated intramyocellular lipid levels yet do not display insulin resistance (27); an increased oxidative capacity of skeletal muscle in these individuals may provide a protective effect lacking in insulin-resistant subjects. Because FFA uptake into muscle is impaired in type 2 diabetes patients even when removed from the in vivo environment, thiazolidinediones may improve insulin sensitivity by modulating FFA uptake, intracellular transport, and β-oxidation, thereby mimicking the effects of training (26).

In summary, we have shown that muscle FFA uptake is, in part, protein mediated and acutely affected by insulin and that, in muscle from type 2 diabetic subjects, the protein-mediated component was decreased and acute insulin regulation of FFA uptake was impaired. We also showed that thiazolidinedione treatment increases FFA metabolism in concert with increased expression of the lipid scavenger and transport protein FAT/CD36. The ability of thiazolidinediones to stimulate FFA metabolism in muscle may contribute to enhanced sensitivity of insulin-stimulated glucose metabolism in this tissue in type 2 diabetes observed after treatment.

DISCLOSURES

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