Fatty Acid-Induced Insulin Resistance in Adipocytes*

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ABSTRACT

Elevated serum-free fatty acid (FFA) levels induce insulin resistance in whole animals and humans. To understand the direct mechanism by which FFAs impact insulin-responsive tissue, we have used our previously developed in vitro model of long-chain saturated fatty acids (LCSFA)-induced insulin resistance in adipocytes. In addition to explanted rat adipocytes, we now demonstrate that overnight exposure of 3T3-L1 adipocytes to 1 mM individually of the LCSFA palmitate, myristate, and stearate, leads to an approximately 50% inhibition of insulin-induced glucose transport. Insulin resistance can be accomplished at 0.3 mM palmitate, which is within the range of palmitate found in diabetic and obese individuals. This inhibition was noted within 4 h of exposure to FFA, which is comparable to in vivo lipid infusion studies. Initial LCSFA-induced resistance is specific to glucose transport and does not affect insulin stimulation of glucose incorporation into glycogen. In 3T3-L1 adipocytes overexpressing the EGF receptor, LCSFA exposure also specifically inhibited EGF-induced GLUT4-mediated glucose transport, but not EGF-induced glycogen synthesis. We find that LCSFA treatment did not impair insulin stimulation of GLUT4 translocation or exofacial presentation on the cell surface as determined by trypsin accessibility. Our results suggest that the initial direct effect of elevated LCSFA is to impair activation of GLUT4 transporter activity and that this effect is specific for glucose transport. (Endocrinology 138: 4338–4345, 1997)

INSULIN resistance, the state in which peripheral tissues demonstrate impaired glucose uptake in response to insulin, is an early predictor of noninsulin-dependent diabetes mellitus (NIDDM) (1, 2). Insulin resistance and NIDDM are both highly correlated with obesity; in fact, weight reduction often leads to increased insulin sensitivity by peripheral tissues (3). A mechanism by which obesity may lead to insulin resistance is through the elevated levels of plasma free fatty acids (FFA) noted in patients with obesity and NIDDM (1, 4–8). This hypothesis has been supported by experiments in which increased dietary FFA induced insulin resistance in explanted adipocytes, muscle, and liver (9–11). Recently, whole-body glucose uptake, glycogen synthesis, and glucose oxidation was shown to be impaired with elevation of plasma FFA levels through lipid infusion (12, 13). However, elevated FFA levels may exert multiple negative effects, depending on the target tissue, and may also reflect secondary, compensatory signals in the whole animal. Therefore, the underlying intracellular signaling mechanisms that are affected by elevated FFA levels are best deciphered in vitro.

Explanted rat adipocytes and 3T3-L1 adipocytes are established in vitro models for studying the signaling mechanism required for insulin-inducible glucose uptake and storage. We have previously demonstrated that exposure of rat adipocytes to certain fatty acids, specifically long-chain saturated fatty acids (LCSFA), resulted in a biphasic response over time (14, 15). Initially, palmitate treatment of rat adipocytes leads to an increase in GLUT4-mediated glucose transport, which is followed by insulin insensitivity for glucose transport following a 4-h exposure to palmitate. Furthermore, we have previously been able to demonstrate a separation of signaling pathways for GLUT4-mediated glucose transport from those mediating glycogen and lipid synthesis by expressing various signaling-restricted epidermal growth factor (EGF) receptors in 3T3-L1 adipocytes (16). Our goals, by extending our studies in 3T3-L1 adipocytes and rat adipocytes, are to determine the following: 1) whether fatty acid induced insulin resistance affects only specific actions of insulin; 2) whether EGF receptor-mediated glucose transport and storage are resistant to LCSFA treatment; and 3) how insulin-induced GLUT4-mediated glucose transport is specifically impaired by LCSFA treatment. Herein, we present results that both rat adipocytes and 3T3-L1 adipocytes are most sensitive to palmitate-induced insulin resistance. Furthermore, palmitate-induced insulin resistance in adipocytes is limited only to insulin- and EGF-stimulated glucose transport and does not affect the ability of either insulin or EGF to stimulate glycogen synthesis. Finally, we present evidence that the early mechanism by which elevated FFA levels inhibit glucose transport is by impairment of GLUT4 activation and not GLUT4 translocation.

Materials and Methods

Preparation of isolated rat adipocytes

Isolated adipocytes were prepared from the epididymal fat-pads of ad libitum fed 100–160 g male Sprague-Dawley rats using the collagenase method of Rodbell (17).

3T3-L1 Cell culture and differentiation

A subclone of 3T3-L1 fibroblasts (ATCC) that does not present detectable EGFR after conversion to adipocytes (18) was maintained in DMEM (25 mmol/liter glucose) supplemented with 7.5% BSA and an-
tibiotics (penicillin and streptomycin, 200 kU/liter each) in 5% CO₂, 90% humidity, at 37 C. The cells were differentiated into adipocytes by standard procedures (19). The cells used for experimentation were >80% differentiated (as determined visually). Generation of 3T3-L1 adipocytes stably expressing the full length human epidermal growth factor receptor (EGFR) was described previously (18).

Addition of LCSFA to buffers and media
Fatty acids were added to aqueous solutions using albumin as a physiologic carrier to introduce hydrophobic fatty acids to cells (20). Fatty acid-free (FAF) BSA was prepared by the acid charcoal method of Chen (21) and specified fatty acids were added back to the FAF BSA via celite by the method of Spector and Hoak (22). Fatty acid concentrations were measured using an enzymatic colorimetric kit (Wako Pure Chemical Industries, Osaka, Japan).

Fatty-acid induced insulin resistance
Adipocytes were made insulin resistant by pretreatment with fatty acids as previously described (15). Briefly, cells were incubated with Krebs-Ringer phosphate buffer (KRP) (pH 7.6) containing 10 nm glucose, 1 mm fatty acid, and 1% BSA at 37 C. Control cells were treated identically as treatments except FAF BSA was added to the KRP buffer instead of fatty-acid loaded BSA. The duration of incubation was either 4 h for rat adipocytes or overnight for 3T3-L1 adipocytes. The incubation buffer was removed and cells were resuspended for another hour at 37 C, with KRP (pH 7.0) containing 1 mM buffer was removed and cells were resuspended for another hour at 37 C instead of fatty-acid loaded BSA. The duration of incubation was either 4 h for rat adipocytes or overnight for 3T3-L1 adipocytes. The incubation buffer was removed and cells were resuspended for another hour at 37 C, with KRP (pH 7.0) containing 1 mM pyruvate and 1% FAF BSA. Cells were then washed two times with KRP (pH 7.6) containing 1% FAF BSA and resuspended in appropriate buffers for experimentation.

2-Deoxyglucose transport
2-Deoxyglucose transport in rat adipocytes were performed as previously described (15). Briefly, control and fatty-acid treated cells were incubated for 15 min at 37 C with or without 1 mM insulin, after which 2-Deoxy-2-deoxyglucose was added for 3 min. Cells were separated by centrifugation through dinonyl phthalate oil and counted by liquid scintillation. Nonspecific glucose transport was determined in the presence of 50 μM 2-deoxyglucose, BSA was added to the KRP buffer instead of fatty-acid loaded BSA. The duration of incubation was either 4 h for rat adipocytes or overnight for 3T3-L1 adipocytes. The incubation buffer was removed and cells were resuspended for another hour at 37 C, with KRP (pH 7.0) containing 1 mM pyruvate and 1% FAF BSA. Cells were then washed two times with KRP (pH 7.6) containing 1% FAF BSA and resuspended in appropriate buffers for experimentation.

Rat adipocyte plasma membranes isolation
Plasma membrane enriched fractions were prepared by differential centrifugation and separation on a Ficoll concentration gradient according to the method of McKeel and Jarett (23). Briefly, cells were homogenized, centrifuged at 20,000 × g for 15 min at 4 C. The pellet was resuspended, centrifuged at 1000 × g for 10 min at 4 C, and the supernatant was then centrifuged at 21,000 × g for 20 min at 4 C. The pellet was resuspended and centrifuged at 58,000 × g for 30 min at 4 C on a Ficoll gradient. The plasma membrane containing band was removed and pelleted by centrifugation at 21,000 × g for 30 min at 4 C. The pellet was resuspended and frozen at −70 C for immunoblotting and glucose transport assays. The purity of plasma membrane fractions was verified by measuring 5’ nucleotidase as previously described (24). Our results indicate that the low density microsomal fractions contain only low levels of 5’ nucleotidase (10%, 6%, 6%, and 8% of the corresponding plasma membrane fractions in control untreated, control plus insulin, palmitate and palmitate plus insulin, respectively).

Plasma membrane associated glucose transport
2-Deoxyglucose transport was measured in plasma membranes according to the filter assay method of Weber et al. (25). Briefly, filters (Type GS, 0.22 μm, Millipore, Bedford, MA) were soaked in ice cold stopping solution (0.1 mM phloretin, 0.1 mM 2- and 1-glucose in TES (0.25 mM sucrose, 10 mM Tris-HCl, pH 7.6, 1 mM EDTA) for 30 min. Plasma membranes were sonicated for 10 sec and 30–50 μg of plasma membranes were added per sample in 30–50 μL TES buffer and equilibrated with an equal volume of 0.2 mM D- and L-glucose in TES at room temperature for 30 min. The membranes are then pulsed with an equal volume of pulsing solution ([3H]-L-glucose and [14C]-D-glucose, 1.0 μCi per sample of each sugar, 0.1 mM final concentration for each). Initial rates of glucose transport were measured by stopping transport after 3 sec with ice-cold stopping solution. The stopped sample was placed on ice and filtered within 3 min. The filter was immediately washed three times with ice-cold stopping solution. The filters were dried, treated with 1 M NaOH, and counted by liquid scintillation. Nonspecific glucose transport is determined by the [3H]-glucose counts.

Immunodetection of GLUT4 translocation and insulin receptor autophosphorylation
Plasma membrane enriched fractions were separated by SDS-PAGE and transferred onto Immobilon-P membrane (Millipore). For detection of GLUT4, the membrane was probed with rabbit polyclonal antibodies against GLUT4 (East Acres Biologicals, Southbridge, MA) and visualized with antirabbit antibody conjugated to alkaline phosphatase (Bio-Rad, Richmond, CA). For determining insulin receptor autophosphorylation in rat adipocytes, the membrane was probed with mouse monoclonal antiphosphotyrosine antibodies (UBI, Lake Placid, NY) and visualized with antirabbit antibody conjugated to alkaline phosphatase. The insulin receptor was identified on the immunoblots on the basis of its molecular weight. To determine insulin receptor autophosphorylation in 3T3-L1 adipocytes, the insulin receptor was immunoprecipitated using mouse monoclonal anti-insulin receptor antibodies (Oncogene Research Products, Uniondale, NY) as described previously (18) before SDS-PAGE and subsequent immunoblotting, as above.

Glycogen synthesis assay
Incorporation of glucose into glycogen was measured essentially as described previously (26). After cells were serum-starved in DMEM supplemented with 0.2% BSA (2 h), the medium was replaced with Buffer A (25 mM Tris-HCl, pH 7.5, 140 mM NaCl, 1.7 mM KCl, 1 mM CaCl₂, 1.47 mM KH₂PO₄, 0.8 mM MgSO₄, 0.2% BSA), Insulin (100 nM) or EGF (25 nM) was added and the incubation continued for 1 hr at 37 C. [U¹⁴C]-glucose was added, and the incubation was continued for 2 h. Assays were terminated by washing with ice-cold PBS and immediately addition of 0.5 mM NaOH to lyse the cells. Lysates were spotted onto filter paper and were washed three times with ice-cold 67% ethanol and once with acetone. The remaining label on the filter paper was detected by scintillation counting.

Trypsin digestion of cell surface GLUT4
Adipocytes were trypsinized according to the method of Czech and Buxton (27). Briefly, control and palmitate-treated cells were treated with or without 10 mM insulin for 15 min at 37 C, then 2 mM potassium cyanide for 20 min TPCK-treated trypsin (Sigma Chemical Co., St. Louis, MO) was added (1 mg/ml final concentration) for 30 min at 37 C. At the end of the digestion period, soybean trypsin inhibitor was added (2 mg/ml final concentration) and the cells were quickly washed three times with KRP containing 20 mM HEPEs (pH 7.4), 1 mM pyruvate, 1 mM trypsin inhibitor, and 2% albumin before homogenization and membrane preparation.

Statistical analysis
Results are expressed as means ± SEM. Comparisons between groups were assessed using a paired, Student’s t test. P < 0.05 was considered statistically significant.

Results
LCSFA cause insulin resistance
Previously, we demonstrated that prolonged treatment of rat adipocytes for 4 h with 1 mM palmitate induces an im-
paired glucose transport response to insulin (15). We now determined if prolonged exposure to other LCSFA, myristate or stearate, would have similar effects on insulin-induced glucose transport in rat adipocytes. After 4 h exposure of rat adipocytes to palmitate or stearate, there was a significant decrease in insulin-inducible glucose transport relative to control (43 and 22 percent, respectively). However, there was no significant difference between control cells and myristate-exposed cells with respect to insulin-induced glucose transport (Fig. 1A). In 3T3-L1 adipocytes palmitate, myristate, and stearate each inhibited insulin-induced glucose transport by 60%, 43%, and 46%, respectively (Fig. 1B). This inhibition by palmitate was noted for insulin concentrations down to 1 nM, below concentrations at which insulin interacts with the IGF-1 receptor. The increased length of incubation from 4 h for rat adipocytes to overnight (~16 h) for 3T3-L1 adipocytes likely accounts for the differences between the effects of LCSFA observed for rat adipocytes vs. 3T3-L1 adipocytes.

Given the higher sensitivity of adipocytes to elevated palmitate levels, we focused on that particular LCSFA. Significant inhibition of insulin-induced glucose transport was achieved with approximately 0.3 mM palmitate (Fig. 1C), a concentration in the range noted in diabetic and obese individuals (15). The inhibitory effect of prolonged palmitate exposure on insulin-induced glucose transport was detectable after 4 h for both rat adipocytes and 3T3-L1 adipocytes (Fig. 2, A and B). The differences in insulin stimulation of glucose transport in rat adipocytes under fatty acid free (control) and palmitate treatment conditions over time were determined to be statistically significant by using pairwise analysis (P < 0.05). The time of onset of insulin resistance was comparable to those found in whole-body lipid infusion studies (12, 13).

**Insulin-induced incorporation of glucose into glycogen is not impaired by palmitate**

*In vivo* studies using lipid infusion experiments have demonstrated that increased plasma FFA concentrations after 4 h impaired not only insulin stimulation of glucose transport but also glycogen synthesis and glucose oxidation (12, 13). Therefore, we determined if palmitate-induced insulin resistance was restricted to only glucose transport or if it had a global effect on other aspects of insulin signaling, such as glycogen synthesis. After overnight treatments of 3T3-L1 adipocytes with palmitate, insulin stimulated glucose transport was reduced approximately 60 percent as previously demonstrated. However, there was no detectable difference in the ability of palmitate-exposed vs. control cells to incorporate glucose into glycogen in response to insulin (Fig. 3). Palmitate also did not impair insulin stimulation of glycogen synthase in rat adipocytes (data not shown). This suggests that the initial direct effects of palmitate-induced insulin resistance is restricted to stimulation of glucose transport and not induction of glucose storage.

**EGF-induced glucose transport is inhibited by palmitate**

We have previously demonstrated that 3T3-L1 adipocytes that overexpress the EGF receptor were able to transport glucose in an EGF-dependent manner (18). While EGF stim-

![Fig. 1. Effect of LCSFA on glucose transport in rat and 3T3-L1 adipocytes. Rat adipocytes (A) and 3T3-L1 adipocytes (B) were exposed to 1 mM (final concentration) of palmitate (P), myristate (M), stearate (S), or fatty acid free media (C) to induce insulin resistance as described in Materials and Methods. To measure the dose response of palmitate (C), 3T3-L1 adipocytes were exposed to various concentrations of palmitate to induce insulin resistance. Cells were then treated with or without (+/−) insulin (1 nM for rat adipocytes; 100 nM for 3T3-L1 adipocytes) for 15 min. Glucose transport was performed as described in Materials and Methods. All points were derived from three to five separate experiments performed in triplicate. Results are shown as the mean ± SEM. *Significantly different from FAF-treated control cells stimulated with insulin (P < 0.05).
ulated translocation of GLUT4 to the plasma membrane as effectively as insulin in these adipocytes, EGF failed to stimulate phosphorylation of IRS-1 and may therefore induce glucose transport by a mechanism different than insulin. With this in mind, we determined if EGF stimulation of glucose transport was also affected by long-term exposure to palmitate. Palmitate treatment reduces both EGF- and insulin-induced glucose transport relative to untreated cells (Fig. 4). Furthermore, palmitate treatment appeared to be specific for EGF-induced glucose transport, similarly to insulin-induced responses, as EGF-induced glycogen synthesis was only marginally affected with palmitate treatment (11.11 ± 2.12% inhibition relative to FAF treatment, n = 2 in quadruplicate) compared with glucose transport. As our previous findings had demonstrated a divergence of the signaling pathways for glucose transport from glycogen and lipid synthesis at the level of the EGF receptor (18), these findings would suggest that palmitate negatively affects events further down the signaling pathway.

**Autophosphorylation of insulin receptor is not affected by palmitate exposure**

The above data suggested that the palmitate-induced insulin resistance was restricted to later steps in the glucose transport signaling pathway. Previously, we had demonstrated that palmitate treatment did not affect insulin binding to its receptor (15). Therefore, we predicted that palmitate-induced insulin resistance would not affect early signaling events such as autophosphorylation of the insulin receptor upon ligand binding. Consistent with our hypothesis, the ability of the insulin receptor to undergo tyrosyl autophos-
Phosphorylation was not impaired by long-term exposure of rat adipocytes to palmitate (Fig. 5, top panel). This has been confirmed by specific immunoprecipitation of insulin receptors from palmitate-treated 3T3-L1 adipocytes (Fig. 5, bottom panel) and rat adipocytes (data not shown). Densitometric analyses of the immunoblots of insulin receptors indicated that insulin stimulated 4.6 ± 1.8-fold and 2.7 ± 1.3-fold increases in insulin receptor tyrosyl autophosphorylation for palmitate and control treated rat adipocytes, respectively (n = 3), and 8.1 ± 2.4-fold and 7.3 ± 0.6-fold increases for palmitate and control treated 3T3-L1 adipocytes, respectively (n = 2).

Palmitate-induced insulin resistance is not due to glucose toxicity

Previously, we had demonstrated that an immediate response of exposure of cells to palmitate is an increase in GLUT4-mediated glucose transport and insulin receptor autophosphorylation (14). It is plausible that this initial stimulation of glucose transport after a prolonged period would lead to glucose toxicity-related insulin resistance that is due to the increased intracellular concentrations of glucosamine pathway metabolites (reviewed in Ref. 28). To determine if this was the case, we inhibited glutamine:fructose-6-phosphate amidotransferase (GFAT), the first and rate-limiting enzymatic activity in the glucosamine pathway, with 20 μM azaserine (29). There was no significant impairment of the ability of palmitate treatment to reduce glucose transport by
insulin stimulation despite the presence of azaserine at concentrations approximately 20 times the ED_{50} for inhibiting GFAT (29) (Fig. 6).

**Palmitate does not impair GLUT4 translocation**

The effects of palmitate treatment are specific to the ability of either insulin or EGF to stimulate glucose transport, indicating a common point of inhibition by palmitate. GLUT4 is a common end point in both EGF and insulin induced glucose transport (18); therefore, we determined whether insulin stimulation of GLUT4 translocation to the plasma membrane was affected. Palmitate did not impair GLUT4 translocation to the plasma membrane when adipocytes were stimulated with insulin (Fig. 7A). Densitometric analyses of these immunoblots demonstrated no significant difference between control and palmitate treated cells with respect to insulin stimulated GLUT4 translocation to the plasma membrane [9 ± 1.8-fold and a 8.2 ± 1.8-fold, for control and palmitate-treated respectively (n = 3)]. However, the amount of GLUT4, as determined by densitometry of plasma membrane-GLUT4 immunoblots, under basal and insulin-stimulating conditions were actually greater in palmitate-treated vs. untreated controls [1.8 ± 0.4-fold and 1.4 ± 0.1-fold increases for basal and insulin-stimulated cells, respectively (n = 3)], further supporting the contention that LCSFA treatment did not impair GLUT4 translocation.

In addition to translocation of GLUT4 to the plasma membrane, insulin stimulation of GLUT4-mediated glucose transport requires other steps, including fusion of GLUT4 containing vesicles to the plasma membrane to display GLUT4 to the exterior of the cell, and activation of the intrinsic transport rate of GLUT4 (reviewed in Ref. 30). Therefore, to assess if GLUT4 in palmitate-treated, insulin-stimulated adipocytes were exposed to the exterior surface of the cell, we used a previously developed technique (27), for distinguishing those GLUT4 accessible to the extracellular space by determining if GLUT4 became susceptible to proteolytic cleavage by trypsin present in the extracellular milieu. The amount of GLUT4 that is susceptible to proteolytic cleavage, as denoted by a shift to faster migrating species, under insulin stimulation is significantly higher in palmitate-treated cells than in control cells (Fig. 7B). This was confirmed with densitometric analysis of the immunoblots demonstrating a 26 ± 2 percent increase in GLUT4 accessible to trypsin cleavage in palmitate-treated cells (n = 3). Therefore, palmitate treatment actually increases the amount of GLUT4 that is accessible to the extracellular space.

Finally, insulin may increase the intrinsic transport rate of GLUT4 at the cell surface, which is manifested in an increased susceptibility of GLUT4 to photoaffinity labeling (31, 32). The intrinsic glucose transport activities of the plasma membranes of these cells were measured to determine whether palmitate was affecting the activation of GLUT4 by insulin stimulation (Fig. 8). The intrinsic glucose transport activity of plasma membranes were lower for palmitate-treated cells than for control cells despite a slight increase in the levels of GLUT4 translocated to the plasma membrane with insulin stimulation of palmitate-treated cells (Fig. 7A).

These results suggest palmitate inhibits activation of GLUT4 by insulin.

**Discussion**

Epidemiologic and experimental studies have pointed to an etiologic role of FFA in insulin resistance (8, 33, 34). To dissect the intracellular mechanism by which fatty acid exposure to insulin-responsive cells would lead to insulin-insensitivity, we previously developed an in vitro model of fatty acid-induced insulin resistance using isolated rat adipocytes (15). We now report that LCSFA’s can also cause insulin resistance in 3T3-L1 adipocytes (Fig. 1B) at physiologic concentrations (Fig. 1C). This effect of LCSFA on insulin signaling is most pronounced with the fatty acid, palmitate (Figs. 1A and B), and occurs after 4 h of exposure, which is comparable to in vivo lipid infusion studies (Fig. 2A and B) (12, 13).

Using 3T3-L1 adipocytes, we also demonstrate that the effects of fatty acids on adipocytes are specific for glucose
transport and do not directly affect insulin stimulation of either glycolytic synthesis (Fig. 3). This further supports previous in vitro findings, which demonstrate that glucose storage is not dependent on hormone-induced glucose transport in adipocytes (16, 35). Previously, we have demonstrated that overexpression of EGF receptors in 3T3-L1 adipocytes confer an EGF-dependent capability to transport glucose in a GLUT4-dependent manner (18). Stimulation of glucose transport by EGF in these adipocytes failed to stimulate a corresponding increase in IRS-1 phosphorylation, suggesting that the mechanism of EGF receptor-mediated stimulation of glucose transport may be different than that of the insulin receptor. However, palmitate treatment of 3T3-L1 adipocytes that overexpressed the EGF receptor also inhibited EGF-induced glucose transport (Fig. 4). Furthermore, the inhibitory effect of palmitate was specific for EGF-induced glucose transport as the glycogen synthesis pathway was not impaired.

The ability of glycogen synthesis to be independent of fatty-acid impaired glucose transport in 3T3-L1 adipocytes suggests that glucose transport is not rate limiting for glycogen synthesis in vitro, and that the impairment of glycogen synthesis and glucose oxidation in vivo, by elevated FFA is via the generation of a distinct extracellular signal such as TNF-α (36–38). This would argue for a further level of complexity in the body’s response to high levels of FFA, one being direct negative modulation of glucose transport, the other being an indirect inhibition of glycogen synthesis, which leads to further deterioration of the insulin response.

We have previously demonstrated that initial exposure to palmitate, like insulin, stimulates basal glucose transport in rat adipocytes by a mechanism that involves both the activation of the insulin receptor kinase and translocation of GLUT4 to the plasma membrane (14). This initial effect of palmitate exposure over long term might lead to decreased insulin sensitivity as a consequence of glucose toxicity through the intracellular accumulation of glucosamine pathway metabolites (reviewed in Ref. 28). However, we believe this mechanism of fatty acid-induced insulin resistance is unlikely as inhibition of glutamine:fructose-6-phosphate amidotransferase, the rate-limiting enzyme action in glucose- induced insulin resistance, by azaserine had no effect on the ability of long-term palmitate treatment to induce insulin resistance (Fig. 6).

A major question is, how do LCSFA impair glucose uptake? The failure of the EGF receptor signaling pathway to bypass fatty acid induced resistance (Fig. 4) suggests that the rate of action for the fatty acids is at a common component in the stimulation of glucose transport by insulin and EGF. We have previously demonstrated that GLUT4 is a common endpoint for both EGF and insulin-induced glucose transport (18). Insulin stimulation of glucose transport requires several steps: translocation of GLUT4-containing vesicles to the plasma membrane; fusion of vesicle membranes to the plasma membrane to present GLUT4 to the exterior of the cell; and activation of GLUT4 (reviewed in Ref. 30). Palmitate did not impair insulin-stimulated translocation of GLUT4 to the plasma membrane (Fig. 7A). Furthermore, it did not prevent the exposure of GLUT4 on the cell exterior and actually led to a slight increase in insulin-resistant adipocytes (Fig. 7B). However, palmitate did impair the ability of GLUT4 containing plasma membrane fractions to transport glucose (Fig. 8). This finding is not surprising as studies have suggested a separate activation step that modulates the intrinsic transporter activity of GLUT4 (31, 32). This finding is consistent with a recent in vitro study that demonstrated decreased photoaffinity labeling (ATB-BMPA) of cell surface GLUT4 from skeletal muscle of high fat fed mice (39), as this reagent labels those cell surface glucose transporters present in a catalytically active state rather than total cell surface glucose transporters (32). Thus, palmitate exposure likely leads to an initial, direct impairment of glucose transport in adipocytes by inhibiting activation of intrinsic GLUT4 transporter activity at the plasma membrane.

By studying the in vitro effects of long-term exposure to fatty acids on adipocytes, we have begun to dissect the intracellular mechanism through which elevated fatty acids may lead to insulin resistance in vitro. The effect of LCSFA, particularly palmitate, is specific for the process of glucose transport and does not reduce the insulin-sensitivity of glycogen synthesis. Furthermore, the ability of glycogen synthesis to be independent of fatty acid-impaired glucose transport in 3T3-L1 adipocytes is suggestive of a separate mechanism by which elevated FFA may lead to impairment of glycogen synthesis in vitro. Herein we have provided mechanistic insight for the initial, FFA-dependent aspect of adipocyte insulin resistance, in that LCSFA appears to alter glucose uptake by directly impairing hormone-stimulated uptake by inhibiting activation of GLUT4. This suggests that FFA levels are a potential therapeutic target for ameliorating insulin resistance.

References