Adipose tissue sensitization to insulin induced by troglitazone and MEDICA 16 in obese Zucker rats in vivo

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Adipose tissue sensitization to insulin induced by troglitazone and MEDICA 16 in obese Zucker rats in vivo. Am J Physiol Endocrinol Metab 284: E795–E803, 2003. First published December 17, 2002; 10.1152/ajpendo.00368.2002.—The putative role played by insulin sensitizers in modulating adipose tissue lipolysis in the fasting state was evaluated in obese conscious Zucker rats treated with troglitazone or β,β‘-tetramethylhexadecanedioic acid (MEDICA 16) and compared with nontreated lean and obese animals. The rates of appearance (Ra) of glycerol and free fatty acid (FFA) in the troglitazone-treated rats. Primary intra-adipose reesterification, and secondary reuptake of plasma FFA in adipose fat were measured using constant infusion of stable isotope-labeled [2H5]glycerol, [2,2-2H2]palmitate, and radioactive [3H]palmitate. The overall lipolytic flux (Ra, glycerol) was increased 1.7- and 1.4-fold in obese animals treated with troglitazone or MEDICA 16, respectively, resulting in increased FFA export (Ra, FFA) in the troglitazone-treated rats. Primary intra-adipose reesterification of lipolysis-derived fatty acids was enhanced twofold by insulin sensitizers, whereas reesterification of plasma fatty acids was unaffected by either treatment. Despite the unchanged Ra, FFA in MEDICA 16 or the increased Ra, FFA induced by troglitazone, very low density lipoprotein production rates were robustly curtailed. Total adipose tissue reesterification, used as an estimate of glucose conversion to glyceride-glycerol, was increased 1.9-fold by treatment with the insulin sensitizers. Our results indicate that, in the fasting state, insulin sensitizers induce, in vivo, a significant activation rather than suppression of adipose tissue lipolysis together with stimulation of glucose conversion to glyceride-glycerol by TZD may promote sensitization to insulin because of decreased diacylglycerol with protein kinase C attenuation (14) or via the Randle cycle, where glucose utilization by muscle is inversely correlated with the use of FFA (35, 36).

This model seems to prevail in the fed state, as judged by the reported decrease in plasma FFA (4, 18) and in FFA flux during the insulin clamp (20) after TZD treatment. However, in the fasting state, at least in rodents, contradictory results were reported. Although Bowen et al. (3) reported a significant decrease in plasma FFA concentrations in fasted Zucker rats treated with troglitazone, troglitazone had no effect on plasma FFA levels of fasted rats made hyperglycemic by glucose infusion (25). Oakes et al. (27) further demonstrated that TZD treatment of postabsorptive obese Zucker rats even resulted in a significant increase in FFA production rates and plasma FFA levels. These results may imply that, in the fasting state, adipose tissue lipolysis by hormone-sensitive lipase could even be increased by TZD treatment, thus apparently refuting the putative antilipolytic effect of TZD (19, 36). However, because total lipolytic rates as reflected by glycerol production were not measured in the above studies, the increased rate of appearance (Ra) of FFA could result from either decreased adipose tissue reesterification, increased lipolytic rates, or both, thus leaving open the question of how TZD affect adipose tissue lipolysis.

Adipose lipolysis and glucose uptake are reciprocally controlled by insulin. Thus lipolysis is suppressed (16), whereas glucose uptake is increased (6). Previous data have pointed to TZD-induced glucose uptake in isolated adipocytes (26) mediated by GLUT4 recruitment (34). However, evidence for the effect of insulin sensitizers on adipose tissue glucose uptake in vivo is still lacking. The infusion of stable isotope-labeled glycerol and palmitate made it possible to measure simultaneously lipolytic (Ra, glycerol) and adipose tissue reesterification rates. The sum of adipose reesterification rates of both lipolysis-derived and plasma-derived fatty acids was used here as an estimate of adipose glucose uptake in vivo in lean and obese animals treated with insulin sensitizers.

The aim of our work is to study the effect of troglitazone, representing the TZD class of insulin sensitiz...
ers (13), and β,β’,-tetramethyldihexadecanedioic acid (MEDICA 16), representing insulin-sensitizing amphi-
carboxylycarnitines (24), on adipose lipolysis in vivo in fasted obese Zucker rats.

METHODS

Animals

Male Zucker obese (fa/fa) and lean (Fa?) rats (Harlan) aged 8–9 wk were treated for 4–5 wk with either troglitazone (200 mg/kg body wt; Parke-Davis) or MEDICA 16 (260 mg/kg body wt) mixed in standard rat diet (55% carbohydrate, 20% protein, 4.5% fat, 12.1% moisture, 3.4% cellulose, 5% ash). There was no difference in food intake between treated groups. The tail artery and vein were cannulated under local anesthesia using 1.5 ml of 2% lignocaine. After catheter placement, animals were released to their cages and allowed to recover for 90 min. Catheter patency was maintained with saline to avoid heparin during measurement of lipolytic rates (21). Animal care and experimental procedures were in accordance with guidelines of the Animal Care Committee of the Hebrew University.

Experimental Protocol

Total body water. Rats fasting for 15 h were injected intraperitoneally with 0.35 g of 10% enriched \( \text{H}_2\text{H}^{18} \)O (Rotem). Later (2 h), after reaching isotopic steady state, \( \text{H}_2\text{H}^{18} \)O enrichment was determined in 0.3-ml tail blood samples, as described previously (22) and modified by us for small animals (24).

FFA, glycerol, and very low density lipoprotein-TG production rates. After recovery, the cannulated animals were infused for 2 h through the tail vein with 98% enriched \([2,2-\text{H}_2]\)palmitate (bound to albumin at a ratio of 6:1; 0.36 \( \mu \text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1} \), Cambridge Isotope Laboratories, Andover, MA) and with 98% enriched \([3\text{H}_3]\)glycerol (0.42 \( \mu \text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1} \), Isotec, Miamisburg, OH) in saline using a Harvard Apparatus syringe pump. Steady-state enrichments of plasma glycerol and palmitate were determined in three blood samples sampled from the tail artery within 90–120 min of constant infusion. Infusion with stable isotopic palmitate and glycerol was then interrupted and replaced with saline for an additional 100 min, during which five blood samples were collected for measuring the decay in the enrichment of palmitate in plasma very low density lipoprotein (VLDL)-TG. The total amount of blood sampled during the study was up to 2.7 ml, and animals with a drop of hematocrit >15% at the end of the infusion period were excluded from the study.

Adipose secondary reesterification rates. Concomitantly with the decay in stable isotope palmitate enrichment, the animals were primed with 29 \( \mu \text{Ci} \) [9,10-\( \text{H}_2 \)]palmitate (HPLC grade; Biosciences, Amersham) followed by its constant infusion (1.87 \( \mu \text{Ci} \cdot 40 \mu \text{mol}^{-1} \cdot \text{min}^{-1} \) for 100 min. The specific activity of plasma palmitate was measured in blood sampled at 30, 60, and 100 min of constant infusion. Animals were killed by decapitation, and samples of the epididymal, subcutaneous, and perirenal adipose tissues were removed quickly and stored in liquid air.

Analysis of Plasma and Tissue Samples

Plasma metabolites and insulin. Plasma TG and insulin were determined using commercial kits (Sorin Biomedica). Glucose was measured by glucose test strips using an Elite glucometer (Bayer). Plasma FFA composition and levels were determined by GC analysis of respective methyl esters (24) with heptadecanoic acid as an internal standard. The ratio of palmitate to total FFA in plasma amounted to 0.36, and no significant differences were found between the various groups of animals.

\( ^{18} \text{O} \) enrichment. \( ^{18} \text{O} \) enrichment in body water was analyzed using isotope ratio mass spectrometry (MAT 252). Plasma palmitate and glycerol isotopic enrichment. Plasma [\( ^{3}\text{H}_3 \)]glycerol enrichment was determined by subjecting 100-\( \mu \)l plasma samples to barium hydroxide-zinc sulfate precipitation followed by passing the supernatant through a mixed cation/anion exchanger. The eluate was collected in reaction vials and evaporated to dryness. Glycerol was derivatized to its t-butylidimethylsilyl derivative and subjected to GC-MS. Plasma free and esterified palmitate were extracted with 5 ml of isopropanol-heptane-2 N \( \text{H}_2\text{SO}_4 \) at a ratio of 40:10:1. The upper heptane phase was separated and further extracted with 1 ml of alkaline 70% methanol, followed by extraction of the alkaline methanol with heptane. The heptane phases consisting of plasma TG were combined. The alkaline methanol containing the free fatty salt was then acidified and extracted two times with 2 ml of heptane to recover plasma FFA. The plasma TG and FFA extracts were subjected to further purification by TLC (heptane-diethyl ether-glacial acetic acid at a ratio of 157:39:3.9). The purified fatty acids and TG were derivatized to their methyl esters and analyzed by GC-MS.

Isotopic enrichment of t-butylidimethylsilyl glycerol and methylpalmitate was determined by GC-MS analysis using a Quatro II Micromass quadrupole mass spectrometer coupled to a gas chromatograph (15 m DB-1 GC capillary column; J & W Scientific, Folsom, CA). The mass spectrometer was operated in the positive electron impact mode at ionization energy of 70 eV and source temperature of 190°C. Methylpalmitate enrichment was determined by selectively monitoring the 270 (\( M \)) and 272 (\( M + 2 \)) mass-to-charge (m/z) ions. The t-butylidimethylsilyl derivative of glycerol was determined by selectively monitoring the 217 (\( M \)) and 220 (\( M + 3 \)) m/z ions.

Specific activity of plasma [9,10-\( ^{3} \text{H} \)]palmitate. Plasma free palmitate (100 \( \mu \)l) extracted as described above was derivatized with \( \alpha \)-bromoacetoephene. The bromophenyl acetyl ester was purified by an HPLC ODS 5 \( \mu \)m column (255 mm \( \times \) 4.6 mm; Thermo Hypersil, London, UK) using isocratic 83% acetonitrile in water (2 ml/min). The specific activity of eluted palmitate was determined as previously described (21).

Incorporation of [9,10-\( ^{3} \text{H} \)]palmitate in adipose fat. Incorporation of labeled palmitate into adipose TG was determined in adipose samples of 100 mg sonicated for 20 min in 5 ml isopropanol-heptane-2 N \( \text{H}_2\text{SO}_4 \) at a ratio of 40:10:1. The heptane phase was extracted with alkaline methanol and counted for radioactivity. Extraction efficiency amounted to nearly 100%, as verified by adding [carboxyl-\( ^{14} \text{C} \)]tri olein (NEN) to the extraction mixture. No significant differences in label incorporation in the three sampled adipose tissues were observed.

Calculations

\( R_a \), FFA and lipolysis. Rates of appearance of palmitate (\( R_a \) palmitate) and glycerol (\( R_a \) glycerol; in \( \mu \text{mol/min} \)) were calculated using Steele’s equation for steady-state conditions (37), as modified by Bier et al. (2) for stable isotopes.

\[ R_a = \left[ \frac{(\text{IE}_{\text{in}}/\text{IE}_{\text{p}}) - 1}{\times F} \right] \]

where \( F \) is the isotope infusion rate (in \( \mu \text{mol/min} \)), \( \text{IE}_{\text{in}} \) is the isotopic enrichment of the infusion, and \( \text{IE}_{\text{p}} \) is the isotopic enrichment of the tracer in plasma at isotopic steady state.
expressed in mole percent excess. The rate of appearance of FFA (Ra, FFA) was calculated by dividing Rn, palmitate by the ratio of palmitate to total FFA in plasma (found to be 0.36 in this study, see METHODS).

Lipolysis was defined as $3 \times R_n$ glycerol.

Primary reesterification. Primary intra-adipose reesterification rates ($\mu$mol/min) were calculated as described by Wolfe et al. (40) and Campbell et al. (5)

\[
\text{primary reesterification} = (3 \times R_n \text{ glycerol}) - R_n \text{ FFA}
\]

VLDL-TG production. Hepatic VLDL-TG production rate was determined by fitting the decay curve of plasma TG-palmitate enrichment to a single exponential equation: $IE_p = IE_0 e^{-kt}$, where IEp and IEn are isotopic enrichments of plasma TG-palmitate at time t and t = 0, respectively, and k is the fractional turnover rate (11). VLDL-TG production rates ($\mu$mol/min) were calculated as $k \times$ plasma TG pool × 3. Plasma TG pool was determined by multiplying plasma TG by plasma volume.

Plasma volume of lean rats was estimated to be 0.031 ml/g body wt (8), amounting to 0.035 ml/g lean body mass (LBM). To avoid overestimation of plasma volume in obese rats, their plasma volumes were assumed to correlate with their LBM rather than body weights, using a factor of 0.035 ml/g LBM.

Secondary adipose reesterification. Secondary adipose reesterification rates ($\mu$mol FFA/min) were calculated as follows

\[
\text{secondary adipose reesterification} = (A/S/\text{R}_p) \times F_m
\]

where A, is the average counts (dpm/g fat) of [9,10-3H]palmitate incorporated in TGs of the subcutaneous, perirenal, and epididymal adipose tissues sampled at the end of the 100-min infusion period; S, is the steady-state specific activity (dpm/\mu mol) of plasma palmitate; Rp, is the ratio of palmitate to total FFA in plasma; and Fm is total body fat in g.

Glucose conversion to glyceride-glycerol. Glucose conversion to glyceride-glycerol was calculated by dividing the sum of primary and secondary adipose reesterification rates (total adipose FFA reesterification) by six, thus accounting for the three esterified residues of fatty acids/glyceride-glycerol and the two residues of glyceride-glycerol/glycerol. It was assumed that glycerol 3-phosphate is derived from glucose uptake and glycolysis in the fat cell.

Total body water and composition. Total body water (TBW) calculations were based on $^3$H$_2$O dilution measurements (7). LBM was taken as TBW/0.72, whereas body fat was taken as the difference between body weight and LBM (22).

Statistics

All values are presented as means ± SE. Statistical analysis was performed by one-way ANOVA. When significant values were obtained, differences between individual means were analyzed by pairwise multiple-comparison analysis (Student-Newman-Keuls method).

RESULTS

Basal characteristics

Basal characteristics of fasted lean, obese, and obese Zucker rats treated with troglitazone or MEDICA 16 are presented in Table 1. Plasma glucose concentrations were slightly but significantly elevated in obese vs. lean rats and reverted to normal by treatment with either troglitazone or MEDICA 16. Plasma insulin concentrations were increased robustly in obese compared with lean rats. Treatment with troglitazone or MEDICA 16 resulted in a significant decrease of plasma insulin. Plasma TG levels were increased significantly in obese compared with lean rats and significantly decreased by troglitazone or MEDICA 16 treatment. Plasma FFA were elevated significantly in obese compared with lean rats and remained unaffected by treatment with troglitazone or MEDICA 16.

Body weight, weight gain, and body composition of lean, obese, and obese-treated rats are presented in Table 2. Body weight and weight gain were increased

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Table 1. Plasma metabolites and insulin in fasted lean and obese rats treated with insulin sensitizers

<table>
<thead>
<tr>
<th></th>
<th>Glucose, mg/100 ml</th>
<th>Insulin, (\mu)U/ml</th>
<th>Triglycerides, mg/100 ml</th>
<th>FFA, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean</td>
<td>95 ± 4</td>
<td>36 ± 12</td>
<td>37 ± 4</td>
<td>0.62 ± 0.07</td>
</tr>
<tr>
<td>Obese</td>
<td>109 ± 3*</td>
<td>272 ± 34*</td>
<td>267 ± 19*</td>
<td>1.22 ± 0.11*</td>
</tr>
<tr>
<td>Obese + troglitazone</td>
<td>85 ± 3†</td>
<td>100 ± 53†</td>
<td>205 ± 18†</td>
<td>1.21 ± 0.11*</td>
</tr>
<tr>
<td>Obese + MEDICA 16</td>
<td>93 ± 3†</td>
<td>115 ± 29†</td>
<td>163 ± 21†</td>
<td>1.39 ± 0.12*</td>
</tr>
</tbody>
</table>

Data are means ± SE; \(n = 6–11\) rats/study group. FFA, free fatty acid; MEDICA 16, \(\beta,\beta^\prime\)-tetramethylhexadecanedioic acid. Plasma glucose, triglycerides, FFA, and insulin were determined as described in METHODS in 15-h fasted rats. \(P < 0.05\), significant compared with lean (*) and nontreated obese (†).

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Table 2. Body weight, weight gain, and body composition in fasted lean and obese rats treated with insulin sensitizers

<table>
<thead>
<tr>
<th></th>
<th>Weight, g</th>
<th>Weight Gain, g/mo</th>
<th>%</th>
<th>g</th>
<th>LBM, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean</td>
<td>345 ± 8</td>
<td>163 ± 5</td>
<td>12.3 ± 0</td>
<td>43 ± 2</td>
<td>307 ± 7</td>
</tr>
<tr>
<td>Obese</td>
<td>496 ± 15*</td>
<td>238 ± 10*</td>
<td>36 ± 1*</td>
<td>178 ± 3*</td>
<td>318 ± 5</td>
</tr>
<tr>
<td>Obese + troglitazone</td>
<td>519 ± 13†</td>
<td>266 ± 6†</td>
<td>34 ± 2*</td>
<td>175 ± 8*</td>
<td>338 ± 3</td>
</tr>
<tr>
<td>Obese + MEDICA 16</td>
<td>484 ± 10*</td>
<td>250 ± 6*</td>
<td>34 ± 2*</td>
<td>162 ± 9*</td>
<td>322 ± 11</td>
</tr>
</tbody>
</table>

Data are means ± SE; \(n = 6–11\) study group. LBM, lean body mass. Weight, weight gain, and body composition were determined as described in METHODS. \(P < 0.05\), significant compared with lean (*) and nontreated obese (†).
in all obese groups compared with lean controls. Treatment with troglitazone but not with MEDICA 16 resulted in a 10% increase in weight gain. Body fat mass and the percentage of body fat were three- to fourfold higher in obese compared with lean rats. However, LBM was not affected significantly by obesity nor by insulin sensitizers, thus making it possible to refer respective metabolic parameters to the similar LBM of the four study groups.

**Adipose Tissue Parameters Expressed per Animal**

The effects of obesity and treatment with insulin sensitizers on adipose tissue lipolysis and primary reesterification rates were evaluated by studying the rate of glycerol and FFA efflux into the plasma compartment in conscious fasted rats infused with stable isotope-labeled glycerol and palmitate until isotopic steady state was reached. Both glycerol (Fig. 1A) and palmitate (Fig. 1B) reached isotopic steady state in all study groups, thus allowing for measuring production rates by averaging values of plasma samples collected within 90–120 min of constant infusion.

Total body average lipolytic rates of the four study groups are presented in Fig. 2A. Lipolytic rates as reflected by glycerol production rates \(3 \times R_a\) were increased significantly in obese compared with lean rats, in line with the respective increase in adipose bed, and were further significantly increased by treatment with insulin sensitizers under conditions where both fat and LBM remained unaffected by treatment.

Primary reesterification rates of FFA, representing the lipolyzed FFA reesterified back into adipose TGs before escaping the tissue, essentially followed the extent of lipolysis in the four study groups. Thus primary reesterification rates were increased 2.2-fold in obese compared with lean controls and increased a further 2-fold after treatment with troglitazone or MEDICA 16 (Fig. 3A).
Adipose FFA efflux rates (Ra FFA) were increased 2.4-fold in the obese vs. lean rats, in line with the respective increase in adipose bed. Adipose FFA efflux rates were further significantly increased by treatment with troglitazone but not with MEDICA 16 (Fig. 4A).

The Ra FFA-to-Ra glycerol ratio, reflecting the fraction of lipolyzed FFA that escaped intra-adipose reesterification and entered the plasma compartment, amounted to 2.2 ± 0.2, 2.2 ± 0.1, 2.0 ± 0.1, and 1.7 ± 0.1 in lean, obese, troglitazone-treated, and MEDICA 16-treated study groups, respectively, being significantly reduced only by MEDICA 16.

Adipose secondary reesterification rates were measured by sampling radioactively labeled adipose TGs under conditions of steady-state plasma palmitate. Influx of plasma FFA into adipose TGs was increased eightfold in obese compared with lean rats and remained unaffected by further treatment with insulin sensitizers (Fig. 5A). Increased adipose secondary reesterification in obese animals was essentially accounted for by an increase in their fat mass.

Because secondary reesterification of FFA may result from both plasma FFA derived from lipolysis of adipose fat as well as that derived from plasma VLDL-TGs, the fraction contributed by each was estimated by measuring VLDL-TG production rates in the four study groups (Fig. 6). VLDL-TG production was increased robustly in obese compared with lean rats, in line with the higher hepatic availability of adipose-derived FFA (Ra FFA). VLDL-TG production was inhibited by troglitazone and MEDICA 16, in line with the reported hypolipidemic activity of insulin sensitiz-
ers (24 and Table 1). Production rates of VLDL-TG amounted to 42, 23, 10, and 6% of adipose secondary reesterification in lean, nontreated obese, troglitazone-treated, and MEDICA 16-treated obese animals, respectively. Because these rates reflect the maximal possible contribution made by VLDL-TG to adipose secondary reesterification, most secondary FFA reuptake is derived from plasma FFA bypassing the liver.

**Adipose Tissue Parameters Expressed per 100 g Fat**

All of the above results are expressed per animal and therefore reflect both changes in fat mass and intrinsic changes of adipose tissue. The measurement of adipose tissue mass by use of the H$_2^{18}$O dilution technique enabled the expression of results per 100 g fat, thus pointing to changes in adipose tissue intrinsic activities (Figs. 2B–5B).

Lipolysis expressed in this way (Fig. 2B) was found to be decreased 1.7 times in insulin-resistant, nontreated obese rats compared with lean rats. However, treatment of obese rats with either troglitazone or MEDICA 16 resulted in a 1.5-fold increase in lipolytic rates, thus reaching rates similar to lean, non-insulin-resistant rats. Similarly, the primary reesterification rate was reduced 1.7-fold in obese vs. lean rats (Fig. 3B). Treatment of obese rats with troglitazone or MEDICA 16 resulted in a 2.2- to 2.5-fold increase in adipose primary reesterification, reaching values even higher than those observed in lean rats. FFA production rates (Fig. 4B) were decreased a similar 1.7-fold in obese vs. lean rats. However, although in troglitazone-treated rats a 1.5 times increase in Ra FFA could be detected, no significant change was observed in MEDICA 16 rats compared with the obese group.

Adipose secondary reesterification expressed per 100 g fat was increased 1.8- to 2.9-fold in all obese groups compared with lean rats and was not affected by insulin sensitizers (Fig. 5B).

**DISCUSSION**

Adipose tissue is considered to be the main target for TZD. It is well established that these agents are peroxisome proliferator-activated receptor (PPAR)$\gamma$ agonists and that activation of PPAR$\gamma$ leads to adipose conversion into small fat cells. Small adipocytes take up more glucose than larger ones and are more sensitive to the antilipolytic action of insulin (1, 28–30, 36), therefore implying restrained lipolysis and retention of lipolysis-derived FFA. In addition to activation of PPAR$\gamma$, TZD have recently been reported to have a
direct effect on adipose glucose uptake not mediated by PPARγ or adipose conversion (26). These paradigms have been evaluated here in vivo by analyzing the lipolytic flux in obese Zucker rats treated with two nonrelated insulin sensitizers in the fasting state where glucose and fatty acids are derived solely from internal sources.

Total body lipolysis, expressed by three times the glycerol production rates per animal, is increased significantly in obese rats compared with their lean controls, similar to results reported in lean and obese humans (17, 33) and in line with the respective increase in adipose mass. However, contrary to the above paradigm, treatment with troglitazone or MEDICA 16 results in a further increase of 1.7 and 1.4 times in lipolytic flux, respectively, indicating that both agents activate whole body lipolysis. It is worth noting that Ra glycerol as a measure for adipose lipolysis may underestimate the activation of lipolysis by insulin sensitizers, since some of the lipolysis-derived glycerol may escape its export in plasma as a result of adipose glycerol kinase induced by treatment with insulin sensitizers (15). Hence, activation of lipolysis by insulin sensitizers may exceed the degree of activation reflected by Ra glycerol. The increase in adipose lipolysis resulting from troglitazone or MEDICA 16 treatment is surprising in the context of sensitization of adipose tissue to insulin. It may reflect an indirect effect of the insulin sensitizers on the lipolytic cascade not mediated by the insulin transduction pathway. These results in obese rats differ from those reported in humans in whom fasting Ra glycerol is unaffected by either troglitazone (32) or rosiglitazone (23). The difference between Zucker rats and humans may be explained by the lower TZD doses used in humans, differences between species, or both.

Activation of adipose lipolysis by insulin sensitizers (Fig. 2A) results in increased availability of lipolysis-derived FFA for export (Fig. 4A) and for intra-adipose primary reesterification (Fig. 3A) when expressed per animal. An increase in primary adipose reesterification by insulin sensitizers may be ascribed to the higher availability of lipolyzed FFA, to GLUT4 recruitment (34), putative increase in glyceroenogenesis resulting from PPARγ-induced adipose phosphoenolpyruvate carboxykinase (10), and in glycerol 3-phosphate resulting from PPARγ-induced adipose glycerol kinase (15). It is worth noting, however, that primary adipose reesterification calculated by the difference between $3 \times$ Ra glycerol and Ra FFA represents fatty acid esterification of glycerol 3-phosphate derived from glucose or from glycolytic three-carbon precursors, but not fatty acid reesterification of glycerol 3-phosphate derived from glycerol via adipose glycerol kinase activity. The increase in total body primary adipose reesterification of lipolysis-derived FFA is short of compromising the overall increase in adipose lipolysis induced by troglitazone, thus resulting in a 1.5-fold increase in Ra FFA in troglitazone-treated obese rats, in line with the results reported by Oakes et al. (27). However, although the glycerol production rate in MEDICA 16-treated rats increases to values similar to those observed in the troglitazone group, Ra FFA is not significantly affected because of increased primary reesterification, indicating that FFA flux does not represent the true lipolytic flux.

In contrast to the increase in adipose lipolysis, primary adipose reesterification, and FFA export in obese compared with lean controls when expressed per animal, all three are reduced in the nontreated obese animals when expressed per fat mass (Figs. 2B, 3B, and 4B). This apparent decrease may reflect either a change in the intrinsic properties of adipose tissue or a decrease in the surface area of adipocytes and their fat droplets relative to fat content in obese compared with lean controls. Adipose conversion by insulin sensitizers is proposed to restore the intrinsic properties or the ratio of surface area to fat content of adipocytes and lipid droplets (28), thus resulting in restoring to lean values all three parameters expressed per fat mass. However, effects of insulin sensitizer on other properties of adipose cells cannot be excluded.

Adipose secondary reesterification is increased 2.8 times in the obese compared with lean rats (Fig. 5B), and, in contrast to adipose primary reesterification, it remains unaffected by treatment with insulin sensitizers. The lack of effect of insulin sensitizers may perhaps reflect differences in compartmentation of adipose glycerol 3-phosphate for primary and secondary reesterification in treated obese animals and changes in adipose tissue blood flow between study groups. It is worth noting that the presently reported secondary reesterification rates in lean rats are lower than those previously reported by us (21) as a result of overestimated specific activities of plasma palmitate in the previous study resulting from impure [3H]palmitate (American Radiolabeled Chemicals, St. Louis, MO). The present results indicate that the extent of adipose

Table 3. Adipose glucose uptake compared with hepatic glucose production in fasted lean and obese rats

<table>
<thead>
<tr>
<th></th>
<th>Glucose Conversion to Glyceride-Glycerol, μmol/min</th>
<th>Hepatic Glucose Production, μmol/min</th>
<th>Adipose Glucose Uptake, %hepatic glucose production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean</td>
<td>0.9 ± 0.2</td>
<td>13.3 ± 1.0</td>
<td>7</td>
</tr>
<tr>
<td>Obese</td>
<td>2.6 ± 0.4*</td>
<td>21.6 ± 1.0*</td>
<td>12</td>
</tr>
<tr>
<td>Obese + troglitazone</td>
<td>5.0 ± 0.4†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obese + MEDICA 16</td>
<td>4.8 ± 0.3†</td>
<td>22.6 ± 1.0†</td>
<td>21</td>
</tr>
</tbody>
</table>

Data are means ± SE. Rates of hepatic glucose production were as previously reported (24). Rates of glucose conversion to glyceride-glycerol were calculated as described in METHODS. $P < 0.05$, significant compared with lean (*) and nontreated obese †.
secondary reesterification in lean rats is relatively small and amounts to only 20% of the primary reesterification rate.

To date, there is no experimental evidence for activation of adipose glucose uptake in vivo by insulin sensitizers. The amount of glucose taken up by adipose tissue may be deduced by summing adipose primary and secondary reesterification fluxes in terms of glyceride-glycerol (Table 3), assuming limited contribution of adipose glycerol kinase and glyceroneogenesis to the adipose glycerol 3-phosphate pool. Lipolyis-derived FFA and plasma FFA are in fact the only sources of FFA available for adipose tissue reesterification in fasted rats, since adipose lipogenesis is minimal in the fasting state. Indeed, our estimated rates of glucose conversion to glyceride-glycerol expressed per animal in lean and obese rats are very close to the rates of glucose transport measured in vivo using 2-deoxyglucose in fasted lean (12) and obese rats (31). Glucose conversion to glyceride-glycerol is increased threefold in the obese vs. lean rats and is further induced twofold by insulin sensitizers (Table 3). This estimated increase in adipose glucose uptake driven by the increased supply of lipolysis-derived fatty acids is in line with previous results reported in rodents and humans (9, 19, 39), where adipose tissue lipolysis induced by treatment with a highly selective \( \beta_3 \)-adrenergic agonist (CL-316,243) resulted in an increase in whole body glucose disposal because of increased glucose uptake in adipose tissue, with no effect in muscle. The estimated increase in adipose glucose uptake may account for 12 and 21% of hepatic glucose production in obese and MEDICA 16-treated obese rats, respectively, compared with only 7% in nontreated lean controls (Table 3). Hence, adipose tissue may have a significant role in controlling whole body glucose disposal in the fasting state in animals treated with insulin sensitizers.

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REFERENCES


