Rosiglitazone Controls Fatty Acid Cycling in Human Adipose Tissue by Means of Glyceroneogenesis and Glycerol Phosphorylation*

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Control of fatty acid homeostasis is crucial to prevent insulin resistance. During fasting, the plasma fatty acid level depends on triglyceride lipolysis and fatty acid re-esterification within fat cells. In rodents, Rosiglitazone controls fatty acid homeostasis by stimulating two pathways in the adipocytes, glyceroneogenesis and glycerol phosphorylation, that provide the glycerol 3-phosphate necessary for fatty acid re-esterification. Here, we analyzed the functionality of both pathways for controlling fatty acid release in subcutaneous adipose tissue samples from lean and overweight women before and after Rosiglitazone ex vivo treatment. In controls, pyruvate, used as a substrate of glyceroneogenesis, could contribute to the re-esterification of up to 65% of the fatty acids released after basal lipolysis, whereas glycerol phosphorylation accounted for only 14 ± 9%. However, the efficiency of glyceroneogenesis diminished as body mass index (BMI) of women increased. After Rosiglitazone treatment, increase of either pyruvate- or glycerol-dependent fatty acid re-esterification was strictly correlated to that of phosphoenolpyruvate carboxykinase and glycerol kinase, the key enzymes of each pathway, but depended on BMI of the women. Whereas the Rosiglitazone responsiveness of glyceroneogenesis was rather constant according to the BMI of the women, glycerol phosphorylation was mostly enhanced in lean women (BMI < 27). Overall, these data indicate that, whereas glyceroneogenesis is more utilized than glycerol phosphorylation for fatty acid re-esterification in human subcutaneous adipose tissue in the physiological situation, both are solicited in response to Rosiglitazone but with lower efficiency when BMI is increased.

Thiazolidinediones (TZD) are recently developed potent hypolipidemic and antidiabetic drugs. Although TZD target the γ isofrom of peroxisome proliferator-activated receptor (PPAR-γ), a nuclear receptor mainly expressed in adipose tissue (AT) and scantily in skeletal muscles, TZD exert their major effect on insulin sensitivity in skeletal muscles. To clarify this peculiar mechanism, numerous studies have revealed that in addition to their effects on the secretion of some adipocytokines (for a review, see Ref. 2), TZD reduced plasma nonesterified fatty acid (FA) levels, therefore preventing FA from opposing insulin action on skeletal muscle and other insulin target tissues. Indeed, evidence is emerging that elevated plasma FA levels contribute to the pathogenesis of metabolic syndrome (3), and plasma FA is increased at the onset of diabetes in diabetic obese Zucker rats (4). One of the proposed mechanisms is that TZD trigger the differentiation of preadipocytes into mature, small, insulin-sensitive, and metabolically active adipocytes able to clear away excessive FA from the circulation (5), thus resulting in a slight competition of FA with glucose for uptake by skeletal muscles and an enhancement of insulin-mediated glucose uptake (6). Another mechanism for TZD capacity to reduce plasma FA levels has been recently highlighted (7, 8). TZD induce a futile metabolic cycle that increases FA recycling by a re-esterification process in AT, resulting in a decreased output to the blood.

Physiologically, this pathway is activated in lipolytic situations like fasting, when triglycerides (TG) are hydrolyzed to produce glycerol and FA (9). Re-esterification supposedly acts as a retro-control pathway for reducing FA release. For this to occur, glycerol 3-phosphate (G3P) synthesis is required. During fasting, G3P does not significantly come from glucose or from glycerol, since glycerol kinase (GyK) activity is very weak in white AT. An additional G3P-synthesizing pathway, named glyceroneogenesis (GNG), was recently highlighted (10). GNG was first described by Reshef et al. (reviewed in Ref. 11) as the provider of G3P, in the lipolytic situation, from non-carbohydrate substrates like lactate and pyruvate; its key enzyme is the cytosolic isofrom of phosphoenolpyruvate carboxykinase (PEPCK-C), whose gene expression is strongly induced in fasting.

Pharmacologically, in response to TZD, FA re-esterification is enhanced by the activation of both GNG and glycerol phosphorylation (GP) in rodents (7, 8), where both PEPCK-C and GyK gene expression are positively controlled by PPAR-γ ligands in adipocytes (7, 12). The participation of both pathways in human AT was only assumed by means of TZD-induced expression of PEPCK-C and GyK genes (7, 13, 14). In the present work, we appreciated for the first time the functionality of GNG and GP for controlling FA release in cultured explants from human subcutaneous AT (SCAT). Physiologically, GNG was found to be the major pathway involved but with an efficiency that decreased when body mass index (BMI) increased. Pharmacologically, both pathways significantly contributed to the hypolipidemic action of Rosiglitazone (Rosi) but were less responsive to this drug in obese subjects.
EXPERIMENTAL PROCEDURES

Culture of Explants and Monitoring of FA Re-esterification—SCAT was obtained from 16 healthy young women (age 36.3 ± 4.1) with BMI from 20 to 33 (average BMI 27.1 ± 4.2). All patients were undergoing elective surgery at Cochin Hospital (Director Prof. C. Chapron) and had given informed consent. Samples were transported in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 5 mM glucose and then cut into small pieces of about 30–50 mg, distributed in 12-well culture plates with about 400 mg/well, and incubated in 2 ml of Dulbecco's modified Eagle's medium with 5 mM glucose, containing biotin, pantothenate, and antibiotics as described (12) in a humidified atmosphere of 10% CO₂ at 37 °C. Medium was changed every day, with or without 1 μM Rosi for 3 days. At day 3, samples were incubated in glucose- and serum-free Dulbecco's modified Eagle's medium containing 3% (w/v) bovine serum albumin for 4 h before incubation with radioisotopes. Then the study of GNG or GP involvement in FA re-esterification was performed as follows. The medium of each well was replaced with 1 ml of Krebs-Ringer bicarbonate buffer containing 3% fatty acid-free bovine serum albumin (catalog number A6003; Sigma) and either no added substrate or [¹⁴C]pyruvate (1 μCi/ml) or [¹⁴C]glycerol (2 μCi/ml) as precursors of G3P. The isotopic dilution of radiolabeled substrates was kept constant at about 1:130. After 1 h, the incubation medium was discarded for the estimation of lipolytic FA and glycerol. Simultaneously, the corresponding tissue fragments were frozen in liquid nitrogen before lipid extraction according to the simplified method of Bligh and Dyer (15). The subsequent [¹⁴C]pyruvate or [¹⁴C]glycerol incorporation into the lipid moiety was estimated by counting the radioactivity associated with this fraction and was used to appreciate the level of FA that have been re-esterified during the lipolytic process. In the experiments carried out under acute lipolytic conditions, 1 μM isoproterenol was added in the incubation medium. For each SCAT, some explants were frozen at day 3 for the determination of PEPCK-C and GyK activities. Unfortunately, some of these parameters have not been evaluated in some SCAT sample because of their too low quantity. This explains why some values are missing for a given individual (or BMI).

Biochemistry—PEPCK-C and GyK activities were estimated in a postmitochondrial fraction, prepared from frozen AT previously homogenized in 10 mM Tris-HCl buffer, pH 7.4, containing 1 mM dithiothreitol. PEPCK-C enzymatic activity was determined as described by Duff et al. (16) by the acute radioactive method of Chang and Lane (17), with saturating substrate concentration and 1:30 isotope dilution of [¹⁴C]NaHCO₃. GyK activity was measured using the radioactive method of Newsholme et al. (18) with 0.3 mM [³H]glycerol (i.e. nonsaturating substrate concentration). Both reactions were incubated for 30 min at 37 °C. Protein content was determined using the BCA protein assay kit from Sigma. Concentrations of glycerol and FA released in the Krebs-Ringer bicarbonate buffer were monitored using
RESULTS

FA Re-esterification in Human SCAT Involves both Glyceroneogenesis and Glycerol Phosphorylation—FA re-esterification in rodent adipocytes requires G3P synthesis, which can be the result of either induced GNG via PEPCK-C (8) or GP via GyK (7). To estimate whether these pathways were effective in humans, we evaluated both specific activities of key enzymes and their functionality in the same human SCAT samples. 3 days after removal the "explants" being cultured in Dulbecco’s Modified Eagle’s medium in the presence or absence of 140 μM mercaptopicolinate (MP), Medium FA and glycerol concentrations were determined, and the ratio FA/glycerol was calculated. This ratio is <3 when glyceroneogenesis occurs. Results represent the mean ± S.D. of three independent experiments, each performed in triplicate, with SCAT from three individuals with BMI = 27.8 and C, the proportion of FA that have been cleared from the culture medium in the presence of 2.5 mM pyruvate was calculated as follows: (FA0 mM Pyr - FA0 mM Pyr/Glyc), in which Glyc is the concentration of glycerol in the culture medium under control conditions (this concentration remains constant whether pyruvate is provided or not). In B, the proportion of FA that have been cleared from the culture medium in the presence of 1 mM glycerol was calculated as follows: (FA0 mM Gly - FA1 mM Gly)/Glyc and plotted against BMI.

Conventional Statistical Analysis—General statistical analysis was performed with JMP statistics software (SAS Institute, Cary, NC). Correlations were examined by nonparametric Spearman’s rank correlation test. p < 0.05.

Fatty Acid Cycling in Human Adipose Tissue

FIGURE 2. Pyruvate- or glycerol-dependent FA clearance from extracellular medium of cultured human SCAT. A, human SCAT was cultured as described under "Experimental Procedures." Pyruvate was then added or not in the incubation medium for 1 h in the presence or absence of 140 μM mercaptopicolinate (MP). Medium FA and glycerol concentrations were determined, and the ratio FA/glycerol was calculated. This ratio is <3 when glyceroneogenesis occurs. Results represent the mean ± S.D. of three independent experiments, each performed in triplicate, with SCAT from three individuals with BMI = 27.8 and C, the proportion of FA that have been cleared from the culture medium in the presence of 2.5 mM pyruvate was calculated as follows: (FA0 mM Pyr - FA0 mM Pyr/Glyc), in which Glyc is the concentration of glycerol in the culture medium under control conditions (this concentration remains constant whether pyruvate is provided or not). In B, the proportion of FA that have been cleared from the culture medium in the presence of 1 mM glycerol was calculated as follows: (FA0 mM Gly - FA1 mM Gly)/Glyc and plotted against BMI.

Calculation of GNG-dependent FA Re-esterification—Hydrolysis of 1 mol of TG leads to 1 mol of glycerol and 3 mol of FA, thus giving a FA/glycerol ratio of 3 in the extracellular medium. Each glycerol molecule taken up from the extracellular medium for G3P synthesis via GyK would be esterified by three FA, leaving the FA/glycerol ratio unchanged. In contrast, when GNG is activated, G3P is synthesized from extracellular sources, such as pyruvate or lactate, that could re-esterify 3 mol of FA, thus decreasing the FA/glycerol ratio below 3.

We then determined the incorporation of radiolabeled [14C]pyruvate as a glyceroneogenic precursor or of [14C]glycerol for GP into the newly synthesized TG in human SCAT. No exogenous FA was added in the assay buffer to ascertain the occurrence of a re-esterification of FA coming directly from basal lipolysis of preexisting TG in SCAT explants. We used pyruvate that was radiolabeled in C1 exclusively, because the C1 cannot be incorporated into acyl-CoA for FA synthesis. Therefore, [14C]pyruvate is a marker of the G3P moiety of TG that arises from GNG only. In such experimental conditions, dispersed values from 1 to 16 nmol of pyruvate incorporated into TG/h/g of AT were obtained (Fig. 1C). These results confirmed the existence of a GNG-dependent FA re-esterification process in human SCAT, but its efficiency decreased as BMI increased (r2 = 0.31). We ascertained the involvement of PEPCK-C in this process by adding a PEPCK-C inhibitor (19), 3-mercaptopicolinate, during the experiment. 140 μM 3-mercaptopicolinate was able to lower

the glycerol UV method and the acyl-CoA oxidase kits from Roche Applied Science.

GyK specific activities were weaker than those of PEPCK-C, with an average of 4.83 ± 3.96 pmol/min/mg protein (Fig. 1B), partly because of the nonsaturating glycerol concentration used for assaying GyK. When plotted against patient BMI, GyK activities were below 6 pmol/min/mg protein for the major part of the patients, with the exception of two overweight women who exhibited higher values (Fig. 1B). Thus, it seems that PEPCK-C and GyK are not identically efficient in the SCAT of subjects according to their BMI, PEPCK-C being more active in lean people and GyK in some overweight patients.

We then determined the incorporation of radiolabeled [14C]pyruvate as a glyceroneogenic precursor or of [14C]glycerol for GP into the newly synthesized TG in human SCAT. No exogenous FA was added in the assay buffer to ascertain the occurrence of a re-esterification of FA coming directly from basal lipolysis of preexisting TG in SCAT explants. We used pyruvate that was radiolabeled in C1 exclusively, because the C1 cannot be incorporated into acyl-CoA for FA synthesis. Therefore, [14C]pyruvate is a marker of the G3P moiety of TG that arises from GNG only. In such experimental conditions, dispersed values from 1 to 16 nmol of pyruvate incorporated into TG/h/g of AT were obtained (Fig. 1C). These results confirmed the existence of a GNG-dependent FA re-esterification process in human SCAT, but its efficiency decreased as BMI increased (r2 = 0.31). We ascertained the involvement of PEPCK-C in this process by adding a PEPCK-C inhibitor (19), 3-mercaptopicolinate, during the experiment. 140 μM 3-mercaptopicolinate was able to lower
by 40% the amount of pyruvate incorporated into TG (results not shown). The involvement of GyK in FA re-esterification was analyzed by providing \[^{14}C\]glycerol as the precursor of G3P (Fig. 1D). The average contribution of GyK to FA re-esterification was found to be \(2.24 \pm 0.95\ \text{nmol/h/g of AT}\) (i.e. 6-fold lower than that of GNG for lean people although in the same order of magnitude for overweight subjects) (Fig. 1, compare C and D).

**Impact of FA Re-esterification on FA Release in Human SCAT**—We then checked whether the FA re-esterification capacity of human SCAT was related to differences in FA release in the extracellular medium and investigated the respective involvement of GNG and Gyk in this process. Since only FA re-esterification via GNG would result in a reduction in the FA/glycerol ratio (as explained under "Experimental Procedures"), the variation of this ratio could be theoretically used to appreciate the participation of GNG in FA release. We first verified if this ratio was dependent on PEPCK-C activity in three subjects with a BMI around 27 by using the PEPCK-C inhibitor, 3-mercaptopicolinate (Fig. 2A). In control, the FA/glycerol ratio was found to be 1.84 instead of the theoretical value of 3, showing that 1.16 \(\text{mol of FA/mmol of lipolyzed TG had been already cleared from the extracellular medium. In the presence of 3-mercaptopicolinate, this ratio increased to 2.8, confirming that the majority of these 1.16 \mu\text{mol of cleared FA had been re-esterified to TG via GNG in a PEPCK-C-dependent manner. Since this was observed in the absence of added pyruvate, results sug-}
gested that residual intracellular substrates for GNG were still present in the SCAT samples, already allowing re-esterification of about 39% of FA generated during lipolysis. When pyruvate was added in the medium, the FA/glycerol ratio decreased in a concentration-dependent manner (Fig. 2A). At the supraphysiological concentration of 25 mM pyruvate, up to 65% FA had been re-esterified via GNG. This meant that only one-third of FA that came from lipolysis was released in the extracellular medium in our experimental conditions and when pyruvate availability was not limiting.

The amount of FA that could be cleared from the culture medium in response to the addition of 2.5 mM pyruvate (figuring physiological concentration) in comparison with no added exogenous substrate (Fig. 2C) was found to range between 250 and 950 nmol/mol of lipolyzed TG (i.e. between 8 and 31%), with a significant inverse correlation with BMI ($r^2 = 0.57$), as found previously for PEPCK-C activity and for $[^{14}C]_i$pyruvate incorporation into TG. Furthermore, for each subject, the amount of cleared FA was found to be correlated to the proportion of $[^{14}C]_i$pyruvate that had been incorporated into the neosynthesized TG in the presence of 2.5 mM pyruvate ($r^2 = 0.65$) (Fig. 2B).

GyK-dependent FA re-esterification was appreciated by comparing the FA concentration in the extracellular medium after a 1-h incubation of the explants with or without 1 mM glycerol. The latter concentration is 4-fold higher than that obtained during basal lipolysis (about 0.25 mM under our experimental conditions) and somehow mimics the glycerol concentration obtained after isoproterenol-induced lipolysis (3-fold induction as observed in Fig. 6A). Under such conditions, a glycerol-related clearance of 422 ± 264 nmol of FA/μmol of hydrolyzed TG (i.e. from 5 to 23%) was observed in SCAT (Fig. 2D). The elevated S.E. value was due to the very high values obtained with SCAT from two overweight patients.

FA Re-esterification Is a Target for Rosiglitazone Action—We examined whether Rosi stimulated PEPCK-C and GyK specific activities in 16 human SCAT explants and if their FA re-esterification capacity increased in a parallel manner. PEPCK-C activity was increased by an average of 1.63 ± 0.38-fold (Fig. 3A) by a 3-day treatment with 1 μM Rosi. Simultaneously, $[^{14}C]_i$pyruvate incorporation into TG was induced 1.73 ± 0.32-fold (Fig. 3B). Three obese individuals with BMI of 29 and 33 were not responsive to Rosi and were not taken into account for calculating the mean ± S.E. value. We found a highly significant correlation between the increase in PEPCK-C activity and function (i.e. $[^{14}C]_i$pyruvate incorporation) (Fig. 3C, $r^2 = 0.68$). In contrast, GyK activity was mostly induced by Rosi in subjects with BMI from 20 to 27 (range 2.5–9-fold) except for one with a BMI of 29, which showed a 5-fold induction (Fig. 3D). In overweight and obese individuals, a null or weak induction was observed. $[^{14}C]_i$Glycerol incorporation into TG reflected the observed Rosi-induced stimulation of GyK activity (Fig. 3E). In both cases, we found correlation coefficients $r^2$ of 0.65 and 0.53, confirming that both Rosi induction of GyK activity and that of GyK-dependent FA re-esterification were inversely correlated to BMI. Additionally, the increase in GyK activity and $[^{14}C]_i$glycerol incorporation into TG were positively correlated ($r^2 = 0.37$; Fig. 3F). Hence, both GNG and GP are functionally induced by Rosi, GyK being more inducible than PEPCK-C in lean subjects.

Comparative Rate of Both FA Re-esterification Pathways after Rosi Treatment of Human SCAT—After Rosi treatment, the rate of pyruvate incorporated into lipids ranged from 30 to 1 nmol/h/g of AT and still...
significantly decreased inversely proportionally to BMI ($r^2 = 0.45$, Fig. 4A) like it did in control (Fig. 1C). In contrast, the involvement of GP in FA re-esterification changed between the physiological and Rosi-induced states (Fig. 4B). Weak and rather constant in control with an average of 2.24 nmol of incorporated glycerol/h/g of AT (Fig. 1D), the GyK-dependent FA re-esterification tended also to decrease with BMI in response to Rosi ($r^2 = 0.26$, $p = 0.07$) and now ranged from 6 to 1.5 nmol/h/g of AT.

Thus, it seemed that, following Rosi treatment, the contribution of both pathways became of similar importance in overweight women and were both diminishing with increased BMI. In only one-fourth of the individuals (three of them having a BMI below 27 and the fourth one a BMI of 30 perhaps associated with a gynoidic obesity), the contribution of pyruvate-dependent FA re-esterification still remained quantitatively the major one and was 5-fold that of glycerol.

Contribution of the GyK-dependent FA Re-esterification Pathway in the Control of FA Release from Rosi-treated Human SCAT—Glycerol release is usually considered as the physiological index of lipolysis because of the negligible GyK activity commonly observed in human AT. However, when GyK gene expression is pharmacologically induced by Rosi, some of the released glycerol molecules could be used back for FA re-estherification, hence decreasing the amount of glycerol from the culture medium. Indeed, under our experimental conditions, we usually found a decrease in the level of released glycerol in almost all of the Rosi-treated SCAT explants when compared with their respective controls (Fig. 5A). This effect was independent from G3P precursor availability and affected an average of 27.7% of the amount of released glycerol. Thus, lipolysis was found to generate an average of $765 \pm 277$ nmol of glycerol/h/g of AT in control SCAT and of $553 \pm 134$ nmol of glycerol/h/g ($p < 0.2$) in SCAT that was pretreated with Rosi. If these cleared glycerol molecules had been used for G3P production, thus allowing FA re-esterification via GyK, their disappearance from the medium should correlate with an increase in their incorporation into TG of the corresponding Rosi-treated SCAT explants. As shown in Fig. 5B, there is indeed a highly significant correlation ($r^2 = 0.57$) between glycerol clearance and incorporation into lipids in response to Rosi treatment versus control. Then we tried to estimate the number of FA molecules that could have been re-esterified via GyK in response to Rosi treatment by multiplying by 3 the number of cleared glycerol molecules (Fig. 5C). Results suggest that the control of FA homeostasis via Rosi-induced glycerol phosphorylation is important, especially in lean subjects, and confirm that it significantly decreases with increased BMI ($r^2 = 0.54$). In Rosi-treated SCAT from lean individuals, GyK seems to be responsible for sequestering about 1200 nmol of FA/µmol of lipolyzed TG (i.e. 40%) (i.e. about 3-fold more in Rosi-pretreated SCAT than in lean controls) (Fig. 5C, empty circles). However, this effect only concerns about 600 nmol/µmol of lipolyzed TG (i.e. 20%) in overweight subjects (Fig. 5C).

These estimated data corroborate the results obtained when the $[^{14}C]$glycerol incorporation into TG was directly estimated (Fig. 4B) and
clearly confirm the existence of an efficient GyK-dependent FA re-esterification in response to Rosi in lean subjects, which decreases with increased BMI.

Contribution of Glyceroneogenesis to the Control of FA Release from Rosi-treated Human SCAT—The involvement of GNG in the control of FA homeostasis was appreciated, as explained under “Experimental Procedures,” by the calculation of the FA/glycerol ratio in the culture medium after 1 h of basal lipolysis and in the presence of 2.5 mM pyruvate (Fig. 6A). The FA/glycerol ratio was found to be 1.17 ± 0.21 nmol/h/g of AT whatever the BMI (20 –33) of the 12 analyzed individuals, showing that under these experimental conditions, only about 40% (1.17/3) of the FA generated by lipolysis of 1 μM TG had been actually released from control SCAT. This is in agreement with the score of 50 – 65% GNG-dependent re-esterified FA as estimated for three individuals with a BMI of 27 (Fig. 2A). When the SCAT explants were pretreated for 3 days with Rosi, this ratio decreased by half to 0.64 ± 0.18 nmol/h/g of AT, and the percentage of released FA decreased from 40% to about 20% of those generated by lipolysis of 1 μM TG and to only 30% in the presence of 140 μM 3-mercaptopicolinate, confirming the involvement of PEPCK-C. We also verified that, in Rosi-treated explants, as previously observed with controls (refer to Fig. 2A), the FA/glycerol ratio decreased when pyruvate concentration increased in the medium (data not shown). We then selected three individuals with a BMI of 27 to analyze the effect that Rosi had on FA release in an acute lipolytic situation. FA output was significantly decreased by one-third following Rosi treatment, even under isoproterenol stimulation (Fig. 6A). Whereas the total amount of released FA was enhanced

![FIGURE 6. Contribution of glyceroneogenesis in the control of FA release from Rosi-treated human SCAT.](image-url)

A, explants were cultured for 3 days with or without 1 μM Rosi in the presence or absence of 140 μM mercaptopicolinate (MP). The impact of glyceroneogenesis on FA release was then appreciated by calculating the FA/glycerol ratio in the culture medium. Results are mean ± S.D. of experiments carried out with SCAT samples from 12 individuals. The effect of an acute treatment with 1 μM isoproterenol (IPR) was estimated after a 1-h incubation of SCAT samples from three individuals with BMI = 27, each performed in triplicate. B, nmol of FA retained in explants due to Rosi-induced glyceroneogenesis and re-esterification was estimated in percentage of control as follows: (FA_c/Gly_c – FA_r/Gly_r)/FA_c/Gly_c × 100, where c and r represent the concentration in FA or glycerol from the culture medium for control and Rosi-treated explants, respectively. These data are plotted against the fold-increase in PEPCK-C activity induced by Rosi treatment of the corresponding SCAT samples. C, the total nmol of FA that have been retained in SCAT explants after Rosi treatment because of Rosi-induced glyceroneogenesis and re-esterification were estimated by adding the nmol of FA that have been retained in response to Rosi in the presence of 2.5 mM pyruvate (i.e. (FA_c – FA_r)/Gly_r) to the nmol of FA that have been retained in the control in a pyruvate-dependent manner (FA_0 mM – FA_2.5 mM)/Gly_r. Values were then plotted against BMI.
(glycerol release is stimulated 2.4 ± 0.8 by isoproterenol), the Rosi effect was identical in both situations and led to the re-esterification of about 500 nmol of FA/μmol of TG (i.e. one-sixth of the lipolyzed TG).

We estimated the percentage of FA that had been retained in AT because of Rosi-activated GNG by comparing the FA/glycerol ratio before and after Rosi treatment (as explained in the legend to Fig. 6B) and plotted it versus the increase of PEPC-K activity. Results confirm that this estimated effect of Rosi treatment on GNG-dependent decrease in FA output from human SCAT is significantly correlated with the level of PEPC-K induction by Rosi (and thus with pyruvate incorporation into TG, since both are correlated; cf. Fig. 3C). In consequence, this Rosi effect on GNG would be responsible for the 1.2–1.8-fold increase (average 1.5) of the level of FA re-esterified found in control human SCAT and, thus, to a corresponding 1.5-fold decrease in nonesterified fatty acid output.

The respective participation of GNG and GP in the general process that reduces FA release after Rosi treatment is difficult to approach because of the simultaneous effect that Rosi exerts on PEPCK-C and on GyK, thus on glycerol release. This does not allow the calculation of the real lipolytic rate in Rosi-treated SCAT, which is different from the GlyGyK, thus on glycerol release. This does not allow the calculation of the percentage was still increased following the addition of pyruvate and pyruvate concentration. We can conclude that in our experimental protocol the FA/glyceride 40% of the FA arising from lipolysis of stored TG. This was responsible for the decrease in FA release in proportion to the percentage was still increased following the addition of pyruvate and pyruvate concentration. We can conclude that in our experimental protocol the FA/glyceride 40% of the FA arising from lipolysis of stored TG. This was responsible for the decrease in FA release in proportion to the percentage was still increased following the addition of pyruvate and pyruvate concentration. We can conclude that in our experimental protocol the FA/glyceride 40% of the FA arising from lipolysis of stored TG. This was responsible for the decrease in FA release in proportion to the percentage was still increased following the addition of pyruvate and pyruvate concentration. We can conclude that in our experimental protocol the FA/glyceride 40% of the FA arising from lipolysis of stored TG. This was responsible for the decrease in FA release in proportion to the percentage was still increased following the addition of pyruvate and pyruvate concentration. We can conclude that in our experimental protocol the FA/glyceride 40% of the FA arising from lipolysis of stored TG. This was responsible for the decrease in FA release in proportion to the percentage was still increased following the addition of pyruvate and pyruvate concentration. We can conclude that in our experimental protocol the FA/glyceride (23) recently observed in young mice fed a low carbohydrate diet (29) mentioned 2 decades ago that half of the obese human population under study exhibited higher GyK activity in AT than lean subjects. They even observed that these obese individuals who have high GyK activity also had greater difficulties in losing weight.

Earlier studies have showed that PEPC-K and GyK were inducible by Rosi in few human AT samples (7, 13, 30). Here we brought some nuances to these preliminary observations by measuring their respective specific activities before and after Rosi treatment in a greater cohort of human individuals exhibiting various BMI. Very interestingly, we observe that, whereas the Rosi responsiveness of PEPC-K is rather independent of BMI, the response of GyK is very high in lean women but decreases when BMI increases. We also confirm that both enzymes are functionally involved in FA re-esterification in relation to the Rosi stimulation of their respective enzyme activities. Thus, our data show that the Rosi-induced increase in FA re-esterification capacity of human SCAT involves both GNG and GP and that both pathways are more Rosi-responsive in the leanest individuals, GyK being the most inducible. Some of the obese subjects were even no longer responsive to the drug, especially when GP was concerned. Identically, Bogacka et al. (14) reported that the Pioglitazone® treatment of type 2 DM obese patients (BMI = 32) only increased PEPC-K mRNA and not GyK in their adipose tissue. In contrast, Guan et al. (7) reported that GyK mRNA was inducible by Rosi in young animal models of obesity (ob/ob mice and Zucker rats). However, these monogenic models develop their obesity very early and are not representative of the most common form of type 2 DM in humans, which develops later and is from multifactor origins.

From a quantitative point of view, our results clearly show that both pyruvate and glycerol phosphorylation reach rather similar performances after Rosi treatment for most of the population in our study, each pathway contributing to the decrease in FA output at an average of 1200 nmol of FA/μmol of TG in lean subjects and at only about 600 nmol of FA/μmol of TG in overweight patients. In one-fourth of the population only, comprising mainly lean people, the contribution of GNG to FA re-esterification remains the major one (cf. Fig. 4). Hence, whereas it is negligible physiologically, GP is, as is GNG, a significant target for the hypolipidemic action of Rosi in human SCAT. However, both pathways seem to be less responsive to Rosi in obese subjects, GyK...
being far more susceptible than PEPCK-C. This might explain why in some obese type 2 DM patients in which GyK mRNA was not induced by TZD (31), no significant decrease in FA plasma level has occurred (32). The only Rosi responsiveness of GNG (whose efficiency was already diminished) in these obese patients was probably not sufficient for a significant decrease of fasting FA to occur. Nevertheless, other studies reported a significant decrease in the fasting plasma FA level in TZD-treated type 2 DM patients (33, 34). Thus, our results suggest that TZD treatment could be given to lean people who already exhibit insulin resistance or present a high risk of developing type 2 DM (as in having affected first relatives). Such preventive therapies have already been undertaken in the United States to slow the appearance of insulin resistance in women who have previously developed gestational diabetes (35).

Finally, how can we explain why GyK gene expression is no longer Rosi-responsive in obese people? At the molecular level, PEPCK-C and GyK are differently controlled via their PPARγ response element in adipocytes; PEPCK-C gene transcription is increased all along the differentiation process (1), whereas GyK gene transcription is inhibited via nuclear receptor co-repressors (36). TZD induce PPARγ-coactivator 1alpha, whose recruitment to the GyK gene is sufficient to release the co-repressor. Our data could suggest that the expression of PPARγ-coactivator 1alpha would be affected in the SCAT of obese women, as observed in the muscle of type 2 DM patients, where its expression level decreased (37).

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