A futile metabolic cycle activated in adipocytes by antidiabetic agents

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Thiazolidinediones (TZDs) are effective therapies for type 2 diabetes, which has reached epidemic proportions in industrialized societies. TZD treatment reduces circulating free fatty acids (FFAs), which oppose insulin actions in skeletal muscle and other insulin target tissues. Here we report that TZDs, acting as ligands for the nuclear receptor peroxisome proliferator-activated receptor (PPAR)-γ, markedly induce adipocyte glycerol kinase (GyK) gene expression. This is surprising, as standard textbooks indicate that adipocytes lack GyK and thereby avoid futile cycles of triglyceride breakdown and resynthesis from glycerol and FFAs. By inducing GyK, TZDs markedly stimulate glycerol incorporation into triglyceride and reduce FFA secretion from adipocytes. The 'futile' fuel cycle resulting from expression of GyK in adipocytes is thus a novel mechanism contributing to reduced FFA levels and perhaps insulin sensitization by antidiabetic therapies.

Obesity and type 2 diabetes are epidemic in industrialized societies[1]. Metabolic dysregulation is a hallmark of diabetes, but for many years the fat cell was viewed largely as a depository for fuel in the form of fatty acids incorporated into triglyceride (TG)[2]. More recently, the adipocyte has been recognized to play an active role in glucose and lipid metabolism, as well as insulin resistance in the pathophysiology of type 2 diabetes[3]. These functions are mediated in part by secreted proteins, including a variety of polypeptides such as leptin, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), adiponectin/ACRP30/adipoQ/apM1 and resistin[4]. In addition, although lipid storage is increased in obesity, elevated circulating free fatty acid (FFA) levels clearly contribute to insulin resistance[5,6]. This effect of FFA is due, in part, to increased levels of diacylglycerol, which activate protein kinase C and ultimately oppose insulin actions in skeletal muscle and other insulin target tissues[7].

The nuclear receptor peroxisome proliferator-activated receptor (PPAR)-γ is one link between fat cells and insulin resistance. PPAR-γ is expressed at its highest levels in fat cells, and is induced during adipocyte differentiation[8,9]. Moreover, expression of PPAR-γ is necessary for normal adipocyte differentiation[10,11], and ligand-activated PPAR-γ is sufficient to induce adipose conversion of fibroblasts[12]. Although other transcription factors such as CAATT/enhancer-binding protein α (C/EBP-α) can also induce adipogenesis[13], PPAR-γ seems to have a higher role in the hierarchy of adipogenic factors[14]. Like other nuclear receptors, PPAR-γ is activated by specific ligands. Polyunsaturated fatty acids and prostaglandin J2 derivatives can activate PPAR-γ at micromolar concentrations, but the physiological activators of PPAR-γ are as yet unknown[15–17]. However, insulin-sensitizing compounds of the thiazolidinedione (TZD) class, used clinically to treat type 2 diabetes, are high-affinity ligands for PPAR-γ (ref. 18). These PPAR-γ activators are adipogenic, and mice and humans gain weight in response to chronic TZD therapy[19]. As weight gain is generally associated with insulin resistance, the mechanisms by which TZDs improve insulin action remain a puzzle. TZDs might have direct effects on muscle and liver; however, the concentration of PPAR-γ is very low in these tissues, particularly skeletal muscle. As a result, much attention has been paid to the effects of TZDs on the adipocyte. Indeed, TZDs regulate the expression of several adipocyte-secreted polypeptides that affect insulin action, including leptin[20–22], adiponectin[23] and resistin[24].

TZD treatment is also associated with reduced circulating FFA levels[25]. The mechanism by which TZDs lower FFA levels is not well understood. Promotion of adipocyte differentiation would favor TG synthesis and storage. Moreover, TZD induction of CD36 and fatty-acid transport protein (FATP) promote FFA entry[26,27]. FFAs are stored as TGs after coupling to glycerol-3-phosphate. In adipocytes, glycerol phosphate is classically held to be generated exclusively from glycolysis and not from glycerol, due to the lack of glycerol kinase (GyK)[28,29]. The physiological consequences, as stated in Matthews and van Holde's textbook *Biochemistry*, are as follows:

> "Because adipose cells lack the enzyme GyK, some glucose metabolism must occur for TG synthesis to take place—specifically, the formation of dihydroxyacetone phosphate (DHAP), for reduction to glycerol-3-phosphate. Glucose acts as a sensor in adipose tissue metabolism. When glucose levels are adequate, continuing production of DHAP generates enough glycerol-3-phosphate for resynthesis of TG from re-

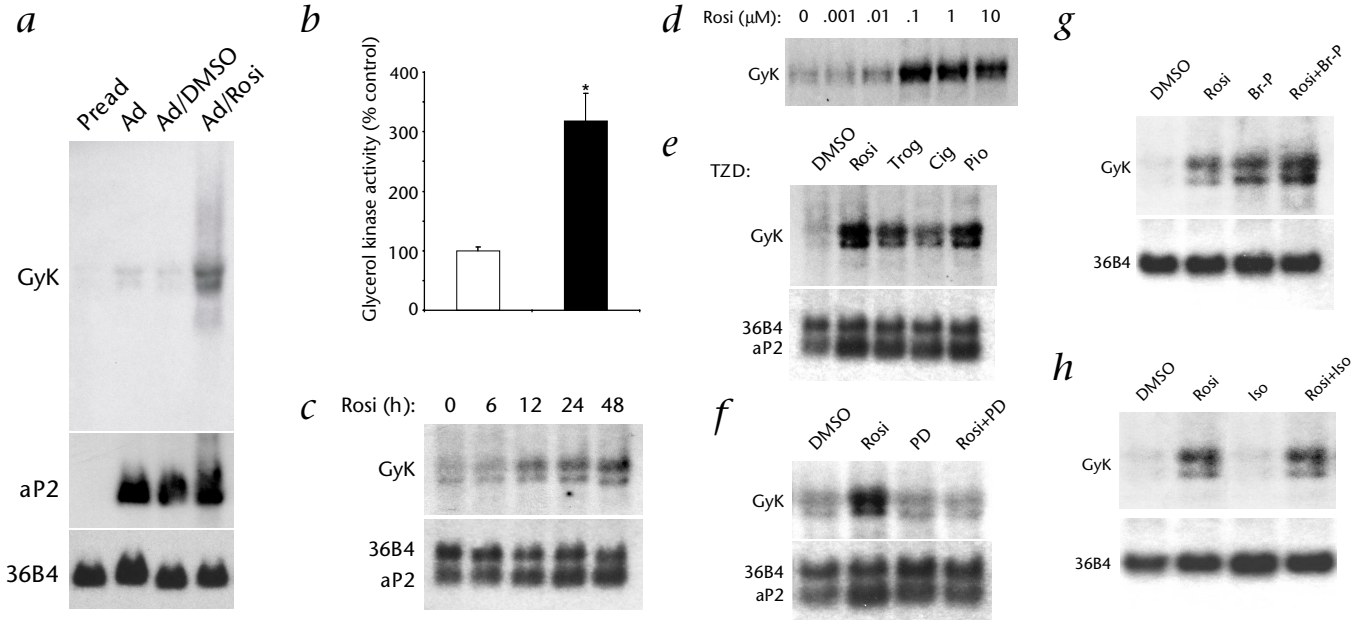

Fig. 1 TZDs increase GyK expression in adipocytes. ***a***, Comparison of GyK, aP2 and 36B4 mRNA levels in 3T3-L1 preadipocytes (Pread), adipocytes (Ad) and rosiglitazone-treated (Rosi) adipocytes. ***b***, GyK enzymatic activity after 48 h treatment of rosiglitazone (■) or DMSO control (□). Results are expressed as the radioactivity of glycerol-3-phosphate per hour (% value of the control). Each bar represents mean ± s.e.m ($n = 6$). *, $P < 0.001$ as compared with DMSO-treated cells. ***c***, Time course of GyK induction by rosiglitazone in adipocytes. ***d***, Rosiglitazone dose-response for GyK induction in adipocytes. ***e***, Multiple TZDs induce GyK in adipocytes. Rosi, rosiglitazone (100 nM); Trog, troglitazone (10 μM); Cig, ciglitazone (10 μM); Pio, pioglitazone (1 μM). ***f***, GyK induction by rosiglitazone is prevented by the PPAR-γ antagonist PD068235 (PD, 100 μM). ***g***, 2-Bromopalmitate (Br-P, 100 μM) induces GyK activity. ***h***, Isoproterenol (Iso, 10 μM) has no effect on GyK activity.

leased fatty acids. When intracellular glucose levels fall, the concentration of glycerol-3-phosphate falls also, and fatty acids are released from the adipocyte for export… to other tissues."[30]

We have made the surprising observation that TZDs markedly induce GyK in adipocytes. As a consequence, the glycerol backbone of TG synthesized in TZD-treated adipocytes preferentially derives from glycerol rather than glucose. Glycerol and FA are the normal products of lipolysis, and the lack of GyK normally prevents adipocytes from recycling the glycerol in a 'futile' cycle of TG hydrolysis and resynthesis. However, abundant GyK in the TZD-treated adipocyte permits this futile cycle to occur, resulting in a marked diminution in FFA release from adipocytes in the basal state as well as following stimulation of lipolysis. This previously unsuspected induction of GyK by TZDs thus switches the internal milieu of the adipocyte to a state of increased TG synthesis and reduced FFA release.

TZDs induce GyK in adipocytes

To understand how exposure of adipocytes to TZDs promotes insulin sensitivity, we searched for genes that are differentially regulated in adipocytes and TZD-treated adipocytes. A comparison of gene expression in 3T3-L1 adipocytes differentiated by constitutively activated PPAR-γ versus adipocytes differentiated by treatment with the TZD rosiglitazone[31] identified GyK as a gene that was expressed at high levels specifically in cells treated with TZD. Northern-blot analysis confirmed that adipocytes differentiated in the absence of TZD expressed little GyK, although aP2 was abundantly expressed, consistent with the adipocyte phenotype (Fig. 1*a*). Remarkably, rosiglitazone markedly induced adipocyte GyK gene expression (Fig. 1*a*). This increase in GyK gene expression was paralleled by an increase in GyK enzymatic activity (Fig. 1*b*). Induction of GyK gene expression by rosiglitazone was half-maximal at 12 hours, and maximal after 24 hours of exposure (Fig. 1*c*). The median effective dose (ED_{50}) for rosiglitazone induction of GyK was between 10 and 100 nM, in agreement with the K_d of rosiglitazone for PPAR-γ (ref. 18) (Fig. 1*d*). Multiple other TZDs, including troglitazone, ciglitazone and pioglitazone, also induced GyK (Fig. 1*e*). Rosiglitazone induced GyK gene expression in the absence of new protein synthesis (data not shown). The non-TZD PPAR-γ ligand GW-7845 also induced GyK (data not shown), and a specific PPAR-γ antagonist, PD068235 (ref. 32) abolished the induction of GyK by rosiglitazone (Fig. 1*f*). Together, these data suggest that the TZDs are acting via PPAR-γ to induce GyK in adipocytes. Although the physiological ligand(s) of PPAR-γ are uncertain, the receptor is activated by fatty acids[15,33]. Bromopalmitate, a poorly metabolized fatty-acid ligand of PPAR-γ (ref. 34), markedly increased the expression of GyK in adipocytes (Fig. 1*g*). The effect of TZDs, however, was not mimicked by isoproterenol (Fig. 1*h*), despite the increase in lipolysis and FFA production induced by this treatment.

TZDs induce adipose GyK *in vivo* and in multiple species

We next explored the effects of TZDs on adipose GyK gene expression *in vivo*. GyK mRNA was markedly increased in adipose tissue of *ob*/*ob* mice treated with ciglitazone (Fig. 2*a* and *b*). The magnitude of GyK induction (3.2 ± 0.1-fold) in adipose was considerably greater than that of aP2 (1.5 ± 0.1-fold), an adipocyte-specific PPAR-γ-responsive gene. Induction of adipose GyK mRNA was dose-responsive and also observed with rosiglitazone (data not shown). A similar increase in GyK expression was observed in lean mice treated with ciglitazone (Fig. 2*c* and *d*). Thus,

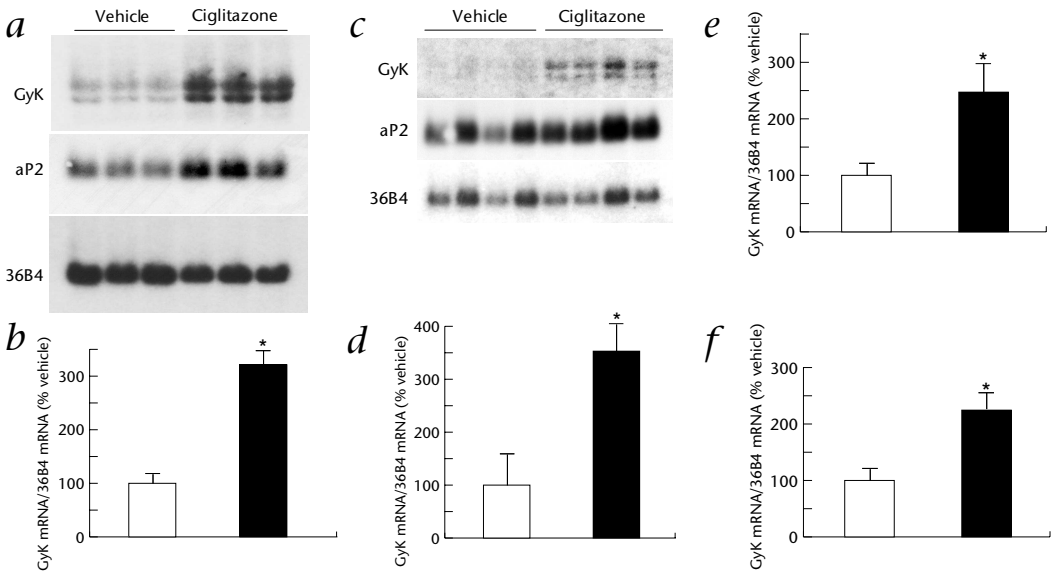

Fig. 2 Ciglitazone induces GyK expression in *ob/ob* mice, lean mice, rats and human primary adipocytes. ***a–d***, Ciglitazone (100 mg/kg/day for 4 d; ■) treatment induces GyK in adipose tissue of *ob/ob* mice (*a* and *b*) and C57BL/6J lean mice (*c* and *d*) versus control (□). Expression of aP2 and 36B4 is shown, and quantification was performed using phosphorimaging with normalization for 36B4 expression. ***e*** and ***f***, Rosiglitazone (4 mg/kg/day for 10 days; ■) increases GyK gene expression versus vehicle (□) in adipose tissue of Zucker diabetic fatty rats (*e*), and cultured human adipocytes (*f*). GyK and 36B4 transcript levels were analyzed by real-time RT-PCR.

GyK induction by TZDs occurs *in vivo* as well as *in vitro*. Rosiglitazone also induced GyK gene expression in adipose tissue of Zucker diabetic fatty rats (Fig. 2*e*), as well as lean Sprague–Dawley rats (data not shown). Furthermore, rosiglitazone treatment induced GyK mRNA to a comparable degree in cultured human adipocytes (Fig. 2*f*), suggesting that GyK is a TZD target gene in adipose tissue of multiple species.

TZD stimulates glycerol incorporation into adipocyte TGs

To determine the consequences of TZD induction of GyK in adipocytes, we performed metabolic studies. 3T3-L1 adipocytes were exposed to rosiglitazone or vehicle for 48 hours to maximally induce GyK, then metabolically labeled with oleic acid, glucose or glycerol (Fig. 3*a*). Oleic acid incorporation into TG increased approximately six-fold in the rosiglitazone-treated cells (Fig. 3*b*). Glucose incorporation into TG increased by ~1.8-fold (Fig. 3*c*), consistent with the previously established ability of rosiglitazone to modestly increase glucose uptake into adipocytes[35]. Although this undoubtedly contributed to the overall increase in TG synthesis, the fold increase was considerably less than that of TG synthesis. Notably, rosiglitazone stimulated glycerol incorporation into adipocytes nearly four-fold (Fig. 3*d*), consistent with the induction of GyK in the cells.

Rosiglitazone reduces fatty acid release from adipocytes

We next studied fatty acid release from adipocytes. Adipocytes were metabolically labeled with oleic acid in the presence of rosiglitazone, and FFA release was assessed after thorough washing in the basal state or during stimulation of lipolysis with isoproterenol (Fig. 4*a*). Albumin and an FFA uptake inhibitor, phloretin, were included in the medium to prevent reuptake of oleic acid, which is stimulated by TZDs (Fig. 3*b*). Rosiglitazone

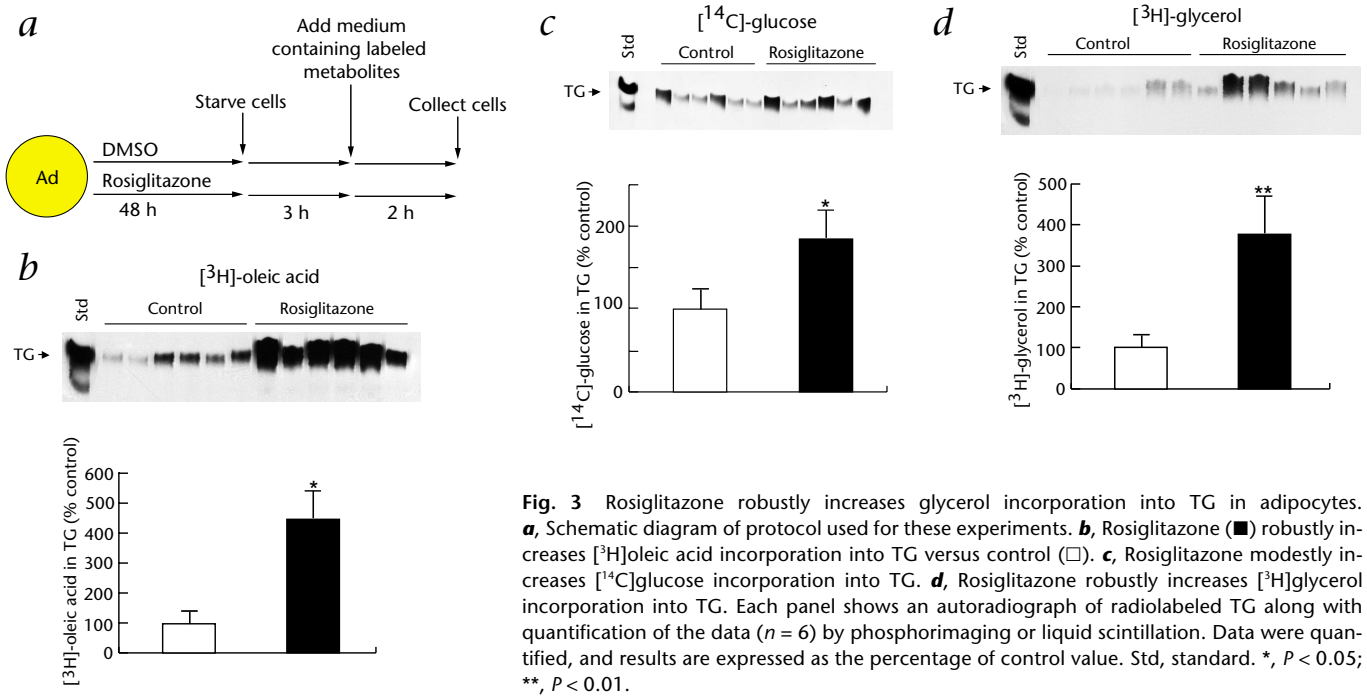

Fig. 3 Rosiglitazone robustly increases glycerol incorporation into TG in adipocytes. ***a***, Schematic diagram of protocol used for these experiments. ***b***, Rosiglitazone (■) robustly increases [^{3}H]oleic acid incorporation into TG versus control (□). ***c***, Rosiglitazone modestly increases [^{14}C]glucose incorporation into TG. ***d***, Rosiglitazone robustly increases [^{3}H]glycerol incorporation into TG. Each panel shows an autoradiograph of radiolabeled TG along with quantification of the data ($n = 6$) by phosphorimaging or liquid scintillation. Data were quantified, and results are expressed as the percentage of control value. Std, standard. *, $P < 0.05$; **, $P < 0.01$.

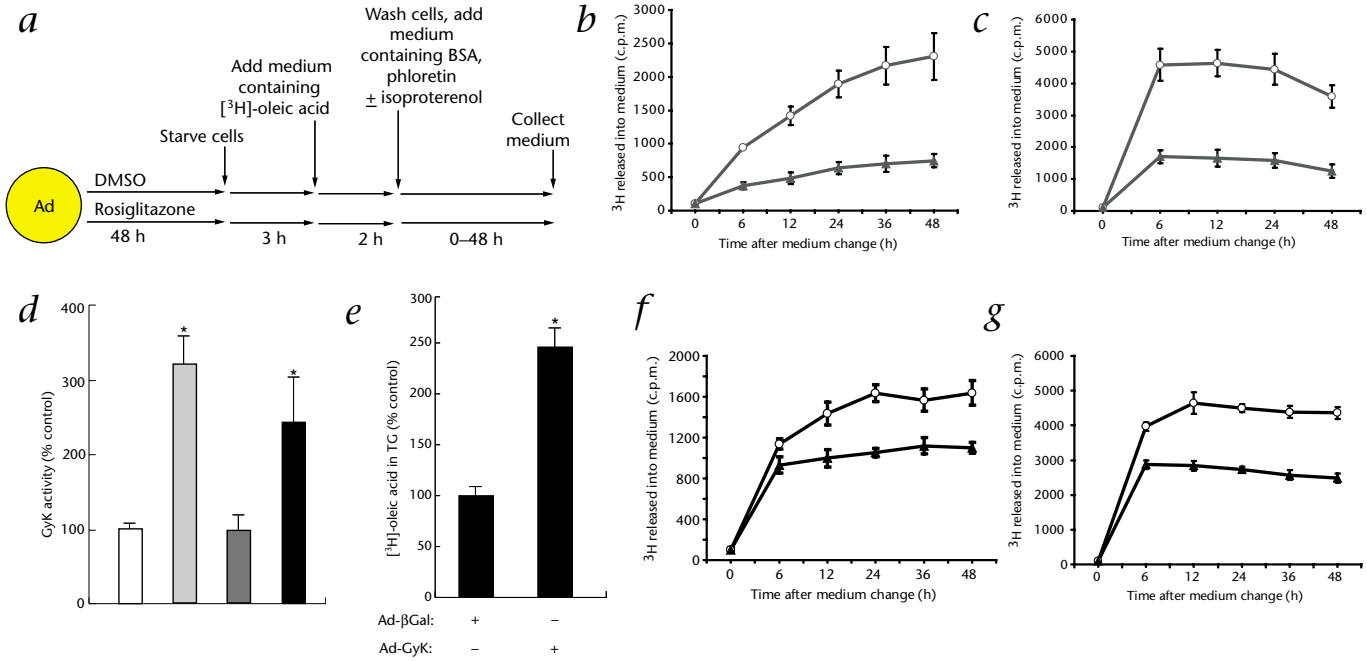

Fig. 4 Rosiglitazone treatment markedly diminishes fatty-acid release from adipocytes, as does GyK overexpression. ***a***, Schematic of experimental design for *b* and *c*. ***b*** and ***c***, Radioactivity of media from 3T3-L1 adipocytes treated with rosiglitazone (▲) or control (○), in the presence (*c*) or absence (*b*) of 10 μM isoproterenol. ***d***, GyK enzyme activity in adipocytes treated with control (□), rosiglitazone (■), infected with β-galactosidase adenovirus (■), or infected with GyK adenovirus (■). ***e***, Incorporation of [^{3}H]oleic acid into TG in adipocytes infected with β-galactosidase adenovirus (Ad-βgal) or GyK adenovirus (Ad-GyK). ***f*** and ***g***, Radioactivity of media from 3T3-L1 adipocytes infected with Ad-βgal (○) or Ad-GyK (▲) in the presence (*g*) or absence (*f*) of 10 μM isoproterenol. For all experiments, $n = 6$. *, $P < 0.01$.

treatment markedly reduced the release of labeled oleic acid from the adipocytes (Fig. 4*b*). This effect is particularly notable considering that the amount of labeled TG in the TZD-treated adipocytes was greater than in the controls (Fig. 3*b*); that is, less was released despite increased starting material. Isoproterenol markedly stimulated FA release from the adipocytes (~5-fold at 6 hours; compare the amount of release in the control cells in Fig. 4*b* and 4*c*). Rosiglitazone impaired isoproterenol-stimulated FFA release. Note that isoproterenol had no appreciable effect upon GyK in the presence or absence of rosiglitazone (Fig. 1*h*). These data suggest that, in adipocytes treated with TZD, the products of both basal and stimulated lipolysis are recycled rather than secreted.

Ectopic expression of GyK in adipocytes mimics TZDs

To assess the contribution of GyK induction to the effects of TZD on adipocyte lipid metabolism, an adenovirus expressing GyK (ref. 37) was used to increase adipocyte GyK activity to approximately the level caused by TZD treatment (Fig. 4*d*). Increased GyK activity stimulated incorporation of oleic acid into TGs to an extent similar to that observed with TZD treatment (Fig. 4*e*). Moreover, GyK expression impaired basal and isoproterenol-stimulated FFA release (Fig. 4*f* and 4*g*), again comparably to TZD treatment. Thus, the effects of TZD on fatty-acid incorporation into TG, as well as on lipolysis, are plausibly explained at least in part by the induction of GyK. Over-expression of GyK is somewhat less effective than rosiglitazone treatment in decreasing fatty-acid release. The greater effect of TZDs is likely due to the coordinated induction of other genes that promote FA incorporation into TG, such as lipoprotein lipase (LPL)[36], CD36 (ref. 27) and FATP (ref. 26). Consistent with this, TZDs have recently been shown to increase expression of the adipocyte glycerol transporter[38], which would function cooperatively with GyK to increase glycerol incorporation into TG.

Discussion

In adipocytes, GyK promotes storage of FA by increasing TG synthesis from precursors that are either imported or recycled within the cell. The latter pathway is uniquely dependent upon expression of GyK. However, induction of adipocyte GyK to physiologically significant levels is noteworthy because it is generally accepted by textbooks of biochemistry, endocrinology and diabetes that GyK is expressed in liver and kidney, but is absent in adipocytes[30,39,40]. This assumption leads to two important physiological principles: 1) in the fed state, adipocytes cannot utilize glycerol and therefore rely on glucose metabolism to synthesize TG (Fig. 5*a*); and 2) in the fasted state, export of lipolysis-generated glycerol and FFA for use as fuel for liver and muscle is unimpeded by a 'futile' cycle of re-utilization of FFA and glycerol to synthesize TG (Fig. 5*b*). The substantial increase in adipocyte GyK in TZD-treated adipocytes markedly changes this physiology.

Our data show a robust increase in glycerol incorporation into TG in TZD-treated adipocytes. Physiologically this suggests that in the fed state adipocytes exposed to TZD can directly convert glycerol to glycerol-3-phosphate, which serves as backbone for esterification with FFA. FFA entry is in turn increased by the TZD induction of LPL, CD36 and FATP (Fig. 5*c*). The other major physiological consequence of GyK activity in adipocytes is the creation of a futile cycle, whereby the products of TG hydrolysis can be recycled into TG within the cell. This is likely to have a major effect on FFA release under lipolytic conditions such as the fasted

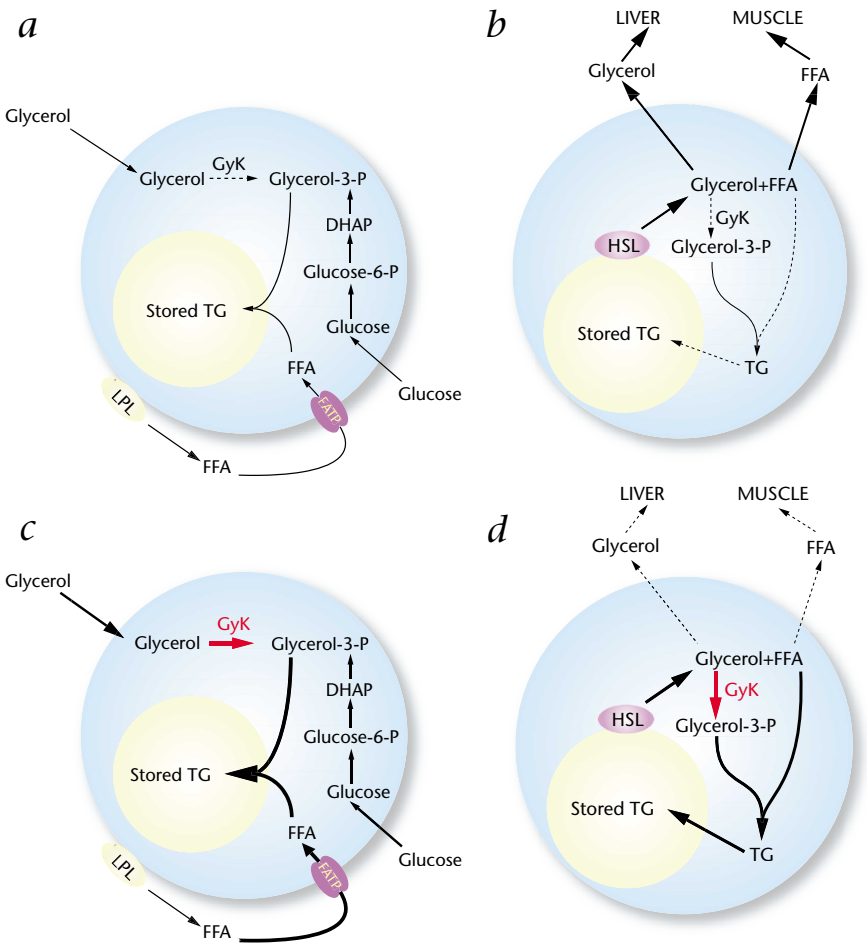

Fig. 5 Model for the effects of TZD treatment on physiology of lipid storage and mobilization in adipocytes. ***a***, Normal adipocytes in fed state. There is little GyK in this condition. Glucose is the main source for TG production. ***b***, Normal adipocytes in fasted state. The stored TG undergoes lipolysis mediated by hormone sensitive lipase (HSL) into glycerol and FFA, the latter are released for utilization in liver and muscle. ***c***, TZD-treated adipocytes in fed state. GyK induced by TZD tremendously increases glycerol incorporation into TG in this condition. As a substrate, FFA is also absorbed into adipocytes for TG production. ***d***, TZD-treated adipocytes in fasted state. The products of lipolysis, glycerol and FFA, are utilized in the futile cycle formed by GyK, and the release of them is inhibited, which decreases the circulating glycerol and FFA.

state (Fig. 5*d*). Indeed, we have shown that FFA release from adipocytes is curtailed even when lipolysis is stimulated by a β-adrenergic agonist which simulates the physiological stimulus for lipolysis during fasting. TZDs have been shown to reduce TNF-α-stimulated lipolysis in adipocytes, although the molecular mechanism was not determined[41]. Induction of adipocyte GyK provides a plausible mechanism by which TZDs reduce the net efflux of glycerol and FFA after stimulation of lipolysis. This would reduce circulating FFA levels and thereby contribute to improved insulin action in target tissues such as liver and muscle[7]. Robust expression of GyK in adipocytes would also tend to reduce circulating levels of glycerol, which has been suggested to contribute to insulin resistance[42].

Models commonly used to calculate lipid turnover and other metabolic parameters from physiological measurements make the assumption that glycerol is not utilized by fat cells for TG synthesis. Our findings suggest that this premise is not valid for animals treated with TZDs, and potentially those treated with other pharmacological agents. GyK induction by PPAR-γ ligands in adipocytes could also be physiologically significant. Metabolic defects associated with GyK deficiency in mice and humans[43] may be caused, in part, by lack of GyK in adipose tissue. Adipose GyK is increased in obesity[44], and this could be due to PPAR-γ activation resulting from elevation of FFA levels. The physiological role of increased adipocyte GyK may comprise a classical feedback loop in which increased circulating FFA causes slightly reduced FFA secretion by adipocytes. However, in obesity the increase in adipocyte GyK level is insufficient to normalize FFA levels, and this effect is clearly overshadowed by the dramatic pharmacological induction of adipocyte GyK by TZDs in obese mice (Fig. 1*g*). This induction likely contributes to decreased FFA levels and, in turn, to improved insulin sensitivity caused by TZDs.

Methods

Northern-blot analysis. 3T3-L1 cells were maintained and differentiated as described[9]. Total RNAs were extracted from adipocytes and epididymal fat pads using TRIZOL (GIBCO–BRL, Grand Island, New York) according to manufacturer's instructions. Total RNA (30 μg) was analyzed for murine GyK expression using a probe digested by *Eco*RI and *Xho*I from plasmid (ATCC #3133528, sequence verified). RNA was also probed for 36B4 mRNA as a loading control, and for aP2 mRNA.

Real-time RT-PCR. Total RNA was isolated from rat epididymal fat and human primary adipocytes (Zen-Bio, Research Triangle Park, North Carolina) using TRIZOL and subjected to DNase digestion followed by RT-PCR (Invitrogen, Carlsbad, California), and mRNA transcripts were quantified by the dual-labeled fluorogenic probe method for real-time RT-PCR, using a Prism 7700 thermal cycler and sequence detector (PerkinElmer/ABI, Norwalk, Connecticut). The primers and probes used in the real-time RT-PCR were the following: rat GyK sense, 5′-GGAGACCAGCCCTGTTAAGCT-3′ and antisense, 5′- GTCCACTGCTCCCACCAATG-3′, and 5′-FAM-CTGATTTCCATGGCAGCCGCG-TAMRA-3′; rat 36B4 sense, 5′- CACCTTCCCACTGGCTGAA-3′, and antisense, 5′- TCCTCCGACTCTTCCTTTGC-3′, and 5′-FAM-AAGGCCTTCCTGGCCGATCCATCTAMRA-3′; human GyK sense 5′-GCAGAAGGAGTCGGCGTATG-3′, and antisense 5′- CCCAACCCATTGACTTCATCA-3′, and 5′-FAM-CGAACCCGAGGATTTGTCTGCCG-TAMRA-3′; human 36B4 sense, 5′- TCGTGGAAGTGACATCGTCTTT-3′, and antisense, 5′- CTGTCTTCCCTGGGCATCA-3′, and 5′-FAM-TGGCAATCCCTGACGCACCG-TAMRA-3′. The cycle number at which GyK transcripts were detectable (CT) was normalized to that of 36B4, referred to as ΔCT. The fold change of GyK expression relative to the vehicle treated group was expressed as $2^{-\Delta\Delta CT}$, in which ΔΔCT equals ΔCT of the TZD-treated group minus ΔCT of the vehicle treated group[45].

GyK enzymatic activity assay. GyK activity was assayed by modification of the method of Noel *et al.*[37]. After treatment with either DMSO or rosiglitazone for 48 h, adipocytes were frozen in liquid nitrogen, then incubated

with extraction buffer (50 mM HEPES pH 7.8, 40 mM KCl, 11 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol) for 30 min on ice, collected and centrifuged at 20,000*g* for 15 min at 4 °C. 10 μg protein was incubated with 50 μl assay buffer (100 mM Tris pH 7.2, 5 mM ATP, 10 mM $MgCl_2$, 100 mM KCl, 2.5 mM dithiothreitol, 4 mM glycerol, with 500 μM ^{3}H-glycerol as tracer) at 37 °C for 3 h. The reaction was terminated by the addition of 100 μl ethanol:methanol (97:3). 50 μl were spotted onto DE-81 Whatman filters (Whatman, Maidstone, England) which were air-dried and washed in water overnight. Radioactivity adhering to the filters was measured by liquid scintillation (Beckman, Fullerton, California).

Triglyceride assay. This method was adapted from a published protocol[46]. After treatment with DMSO or rosiglitazone for 48 h, 3T3-L1 adipocytes were incubated in Kreb's Ringer phosphate buffer (KRPB) (128 mmol/l NaCl, 4.7 mmol/l KCl, 1.25 mmol/l $CaCl_2$, 10 mmol/l sodium phosphate and 50 mmol/l HEPES, pH 7.4) for 3 h, then the medium was changed to KRPB with 5 mM glucose and 100 μM glycerol plus 25 μCi radiolabeled substrates [^{3}H]-glycerol, [^{14}C]-glucose or [^{3}H]-oleic acid) in the presence of DMSO or rosiglitazone and incubated for 2 h at 37 °C. Cells were vigorously washed 3 times in ice-cold 1 × PBS and then collected and homogenized in chloroform:methanol (1:2). Neutral lipids were separated by thin-layer chromatography on Whatman Silica Gel 60A plates in hexane:diethyl ether:acetic acid (80:20:2). Equal amounts of total lipids determined by Triglyceride Reagent (Sigma Diagnostics, St. Louis, Missouri) were analyzed by TLC. The radiolabeled TGs were analyzed by phosphorimaging using Image Quant software.

FA-release experiments. Adipocytes were treated with DMSO or rosiglitazone (100 nM) for 48 h and starved in KRPB for 3 h and the media was changed to fresh KRPB with [^{3}H]-oleic acid (25 μCi/10 cm plate) plus DMSO or rosiglitazone and incubated for 2 h. Cells were washed vigorously with ice-cold 1× PBS 3 times. Fresh media with 2% BSA and 400 μM phloretin (an FA-uptake inhibitor) were added to the cells and incubated for 48 h. The BSA plus phloretin reduced fatty-acid uptake by ~80% (data not shown). Radioactivities of 100 μl aliquots of the media at different timepoints were measured by liquid scintillation.

Adenoviral infection of 3T3-L1 adipocytes. 3T3-L1 adipocytes were exposed to either GyK-expressing adenovirus or β-galactosidase-expressing adenovirus (AdCMV-GlpK and AdCMV-βGal, respectively[37]). After 16 h, the virus-containing media were aspirated, and cells were washed with PBS then cultured in fresh media for 48 h before experimental protocols as described.

***In vivo* experiments**. C57BL/6J lean and *ob*/*ob* mice (Jackson Labs, Bar Harbor, Maine) aged 11 wk, Zucker diabetic fatty (*fa*/*fa*) rats, and Sprague–Dawley rats (Charles River Breeding Laboratories, Wilmington, Massachusetts) aged 9–12 wk were housed (n = 4 per cage for mice and n = 2 per cage for rats) under 12 h light/dark cycles (lights on at 06:00 a.m.), ambient temperature 23 °C, with *ad libitum* access to food and water. Animals were mock-dosed with vehicle for 4 d before actual dosing by oral gavage with ciglitazone in 0.25% w:v methylcellulose (100 mg/kg/day) or vehicle at 05:00 p.m. everyday for 4 d. Rats were administered rosiglitazone in 0.25% w:v methylcellulose (4 mg/kg/d) for 10 d by oral gavage. Epididymal adipose tissues were dissected and total RNAs were extracted for northern-blot analysis. Animal care and procedures were in accordance with guidelines and regulations of the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Statistical analysis. Data are expressed as mean ± s.e.m. Statistical analysis was performed using Student's *t*-test.

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Competing interests statement

The authors declare competing financial interests: see the website (http://medicine.nature.com) for details.

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