In Vivo Multi-Tissue Efficacy of Peroxisome Proliferator-Activated Receptor-γ Therapy on Glucose and Fatty Acid Metabolism in Obese Type 2 Diabetic Rats

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Objective: To identify the disturbances in glucose and lipid metabolism observed in type 2 diabetes mellitus, we examined the interaction and contribution of multiple tissues (liver, heart, muscle, and brown adipose tissue) and monitored the effects of the Peroxisome Proliferator-Activated Receptor-γ (PPAR-γ) agonist rosiglitazone (RGZ) on metabolism in these tissues.

Design and Methods: Rates of [¹⁸F]fluorodeoxyglucose ([¹⁸F]FDG) and [¹¹C]Palmitate uptake and utilization in the Zucker diabetic fatty (ZDF) rat were quantified using noninvasive positron emission tomography imaging and quantitative modeling in comparison to lean Zucker rats. Furthermore, we studied two separate groups of RGZ-treated and untreated ZDF rats.

Results: Glucose uptake is impaired in ZDF brown fat, muscle, and heart tissues compared to leans, while RGZ treatment increased glucose uptake compared to untreated ZDF rats. Fatty acid (FA) uptake decreased, but FA flux increased in brown fat and skeletal muscle of ZDF rats. RGZ treatment increased uptake of FA in brown fat but decreased uptake and utilization in liver, muscle, and heart.

Conclusion: Our data indicate tissue-specific mechanisms for glucose and FA disposal as well as differential action of insulin-sensitizing drugs to normalize substrate handling and highlight the role that preclinical imaging may play in screening drugs for obesity and diabetes.

Introduction

Type 2 diabetes mellitus (T2DM) is a result of systemic disturbances in metabolism characterized by impaired insulin action in peripheral tissues such as liver, muscle, and adipose tissue (1). Recent epidemiological and experimental evidence suggests that there is a close link between the pathogenesis of obesity and T2DM (2). In general, animal models of obesity and T2DM attempt to recapitulate the phenotype of insulin resistance in T2DM, which involves metabolic disturbances in multiple tissues including liver, muscle, white adipose tissue, and brown adipose tissue (BAT), pancreas, among others, and secondary effects on the heart (3).

Skeletal muscle is a major contributor to peripheral glucose disposal, accounting for over 60% of glucose transporter (GLUT)-mediated glucose uptake. Increased supply and/or decreased oxidation of lipids beyond the oxidative capacity lead to accumulation of intramuscular triglycerides (IMTGs), which have been linked to insulin resistance (4). In turn, insulin-mediated glucose uptake is diminished, causing elevated plasma glucose levels. Recent work in BAT, a tissue originally thought to be minimally existent in adults, has sparked interest as a potential therapeutic role in combating obesity and T2DM (5). Like in skeletal muscle, glucose uptake in BAT is mediated by GLUT4 (6); thus, BAT is a tissue of interest in developing insulin-sensitizing drugs. BAT is also a thermoregulator; increased density of mitochondria in BAT produces more energy via substrate oxidation, which is dissipated as heat in the tissue (7). The liver is the central organ for glucose homeostasis (8). Insulin resistance acts as positive feedback by increasing the rate of gluconeogenesis when plasma glucose is already high. The liver is also a major site for fatty acid (FA) oxidation; however, high intracellular lipid content will blunt the oxidative pathway resulting in more storage of FAs and...
eventually release of very low-density lipoproteins, thus increasing peripheral free fatty acids (FFA) levels (9). Finally, a number of studies have focused on interplay among obesity, diabetes, and secondary effects on substrate metabolism in the heart. In previous publications, we have demonstrated that decreased glucose utilization in the Zucker diabetic fatty (ZDF) rat is associated with lowered expression of GLUT4 in the heart, as well as the effect of rosiglitazone (RGZ) to increase glucose utilization as well as FA oxidation in ZDF rat hearts (10,11). In isolated ZDF rat hearts, greater concentration of FA transport proteins at the plasma membrane is associated with greater flux of FA into the tissue (12). However, the two factors influencing increased FA transport (high circulating FFA levels and increased expression of transport proteins) are rarely examined independently.

In light of the highly interconnected and coordinated nature of substrate metabolism, and its failure in disease, an understanding of intrinsic and extrinsic metabolic mechanisms in affected tissues is critical to devising therapeutic approaches for T2DM. Thus, the objective of this work was to characterize in vivo metabolic alterations in multiple tissues affected by T2DM. In addition, the secondary goal of the present work was to assess multi-tissue metabolic efficacy of the PPARγ agonist RGZ. PPARγ agonists of the thiazolidinedione class have been shown to improve whole body insulin sensitivity (13). We have previously assessed the effects of RGZ on myocardial FA metabolism (10). However, the efficacy of PPARγ agonists on both glucose and FA metabolism in affected tissues has yet to be determined in vivo. To that end, in our current work, we aim to, first, assess the metabolic phenotype of glucose and FA metabolism in the liver, heart, skeletal muscle, and BAT of nondiabetic and diabetic rats and, second, assess the effects of RGZ on glucose and FA metabolism in affected tissues.

Methods

All chemicals were purchased from Aldrich Chemical Co. (St. Louis, MO). Radioactive samples were counted on an 8000 γ-counter (Beckman Coulter, Indianapolis, IN). Positron emission tomography (PET) radiotracers [18F]FDG and [11C]Palmitate, metabolic ana-

tabolism in affected tissues.

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Animal models and preparation

Metabolic imaging studies utilized one group of lean Zucker rats and three groups of ZDF: lean (N = 6) and one group of ZDF (N = 6) rats were studied at 14 weeks to characterize alterations in metabolic substrate preference (denoted as “No Treatment” baseline groups); one group of ZDF rats (N = 6) was studied at age of 19 weeks; and one group of ZDF rats (N = 6) was treated with RGZ (4 mg/kg/day; N = 6) for 5 weeks starting at the age of 14 weeks and studied at age of 19 weeks (denoted as “Treatment” group). Small-animal PET was performed on all animals at either 14 or 19 weeks of age as indicated above. A separate group of rats were used to characterize arterial and portal vein (PV) tracer kinetics for use in quantification of the liver dual input function (LDIF) as described in the supplemental material. All animals were fed Purina Constant Nutrition 5008 (Purina, St. Louis, MO) diet throughout this study, with the treated group receiving the RGZ drug in its diet. Animals were fasted overnight prior to imaging. All studies were approved by the Washington University Animal Studies Committee in agreement with the Guidelines for the Care and Use of Laboratory Animals.

Preclinical PET imaging protocol

Dynamic PET acquisition was started after a bolus injection of radiopharmaceutical via tail vein. Rats were anesthetized by inhalation of 2-2.5% isoflurane administered via an induction chamber. Small-animal PET was performed on either the microPET Inveon or Focus-220 (14) (Siemens Inc., Malvern, PA). Scanners were cross-calibrated to ensure reliability of the data. Body temperature was maintained at 37°C by a circulating water blanket and heat lamp. In addition, heart and respiratory rates were monitored throughout the imaging session. The session began with a 20-min acquisition with [11C]Palmitate (22.2-29.6 MBq) to quantify tissue FA metabolism followed by a 60-min scan with [18F]FDG (18.5-29.6 MBq) to quantify tissue glucose metabolism. During each imaging session, five to six arterial whole-blood samples were taken from the femoral artery to measure plasma glycosylated hemoglobin (HbA1c) and glucose (5 μL), insulin (5 μL), and FA (FFA; 20 μL) levels, as well as to correct for the presence of 13CO2 metabolites (15).

Image processing

Images from PET scans were reconstructed using filtered back-projection algorithm. The images from all the frames were summed into a single image, and regions of interest (ROIs) were drawn on the right lobe of the liver to obtain the liver time activity curve (TAC) (5), on left intercostal muscle to obtain the muscle time activity curve (5), and on interscapular fat to obtain the BAT TAC. The ROIs from the summed image of first tracer imaging were subsequently transferred to the successive image scans with other trac- ers to maintain consistent ROIs.

Image quantification

In reporting tissue metabolic parameters, a distinction is made between intrinsic and extrinsic measures. Intrinsic metabolic parameters refer to the intrinsic capacity of the tissue to metabolize a given substrate and are reported as rates, whereas the extrinsic meta-
bolic parameters take into account peripheral concentration of a given substrate to yield the flux into tissue.

Derivation of the input function

The arterial input function (AIF) was reconstructed using the hybrid input blood sampling (HIBS) algorithm, as described previously (16). HIBS-derived input functions were corrected for 11C metabolites, as described previously (15). To assess liver metabolism, the LDIF was thus a weighted sum of the AIF and the estimated PV kinetics weighted by their respective flow fractions. A detailed description of the LDIF quantification is shown in the Supplementary Methods.

Brown fat and muscle substrate metabolism

FA metabolism. The compartmental model describing [11C]Palmitate kinetics in brown fat and muscle is shown in Figure 1A. The model consisted of one vascular compartment and two tissue compart- ments C1(t) and C2(t), representing the activity in the interstitial and cytosolic space, and slow-turnover lipid pool, respectively. The rate constants denoted by K1 (ml/min/g), k2 (min−1), k3 (min−1), and
k_1 (min^{-1}) represent the forward transport, back-diffusion into plasma, fatty acid esterification (FAE), and lipolysis, respectively. The disadvantage of this model is that the oxidative flux and clearance are lumped into the clearance parameter k_2; the rationale is that both the clearance of [11C]Palmitate and oxidative metabolites of [11C]Palmitate are cleared through the periphery. Higher-order models (three tissue compartments, five parameters) were tested; however, they did not show statistical need. The kinetic parameters k_1, k_2, k_3, and k_4 were determined by optimizing the model against the tissue TAC using a nonlinear least squares approach formulated in MATLAB (MathWorks Inc., Natick, MA). The tracer uptakes and the tissue TAC using a nonlinear least squares approach formulated in MATLAB. The tracer uptakes and fluxes were estimated using steady-state analysis. Here, we report three measures of FA metabolism: K_1, the transport rate constant; FAE_{int}, the intrinsic FAE rate; and FAE_{ext}, the extrinsic FAE rate (see Supplementary Methods). Intrinsic measures of FA metabolism, FAE_{int}, represent the uptake rate constant of FA destined for esterification, whereas FAE_{ext} represents the flux destined for esterification.

Glucose metabolism
A three-compartment, three-parameter model, i.e., omitting k_4, was used to describe the kinetics of [18F]FDG, where C_1(t) and C_2(t) represent the unphosphorylated and phosphorylated [18F]FDG compartments, respectively (17). We chose to omit k_4, the dephosphorylation rate constant, when estimating parameters in muscle and BAT due to the very low concentrations of glucose-6-phosphatase, the hydrolyzing enzyme involved in dephosphorylation of G6P (18). To estimate the three parameters K_1, k_2, and k_4, 60 min of [18F]FDG data were optimized against the tissue TAC. For glucose metabolism, we also show three estimates: K_1, glucose transport rate constant; GUR_{int}, the intrinsic glucose utilization rate; and GUR_{ext}, the extrinsic glucose utilization rate (see Supplementary Methods).

Cardiac substrate metabolism
A four-compartment model shown in Figure 1B was used to estimate [11C]Palmitate kinetics in the heart as shown previously in treated ZDF rats (10). The model is able to delineate FAE and oxidation pathways. Consequently, intrinsic and extrinsic fatty acid utilization (FAU; FAU_{int} and FAU_{ext}) in the heart is a combination of both oxidized and stored FAs. Measures of glucose utilization in the heart were estimated as described previously (11) using a graphical analysis approach (19) to derive GUR_{int}. In the subsequent data, we will consistently use the labels GUR_{ext} and GUR_{int} to represent the extrinsic and intrinsic glucose uptake rate, respectively, given we have distinguished their meanings in each tissue.

Hepatic substrate metabolism
FA metabolism. The two tissue compartmental model in Figure 1A was used to describe [11C]Palmitate kinetics in the liver. A similar compartmental model was used before to describe kinetics of FA analog tracer in the liver (20). The first tissue compartment C_1 represents the unmetabolized [11C]Palmitate, and the second compartment C_2 represents the esterification of [11C]Palmitate. The rate constants for the compartmental model are denoted by K_1 (ml/min/g), k_2 (min^{-1}), k_3 (min^{-1}), and k_4 (min^{-1}). FAE is defined as is for brown fat and muscle. More detail of the computations can be found in the Supplementary Methods.

Glucose metabolism. To describe [18F]FDG kinetics in liver, several kinetic models were evaluated. Specifically, we evaluated three variations of the typical two-compartment model: (A) a three-parameter (3P) model depicting trapping of [18F]FDG metabolites; (B) a four-parameter (4P) model depicting phosphorylation and dephosphorylation of [18F]FDG and 18FDG6P, respectively; and (C) based on a recent report on potential downstream metabolism of [18F]FDG (21), a five-parameter (5P) model was evaluated to account for metabolism beyond [18F]FDG6P. However, statistical analysis suggested that the standard two-compartment 3P was found to be sufficient to describe [18F]FDG kinetics in the liver (data not shown). Overall, we did not observe significant differences in [18F]FDG metabolism in the liver between lean and diabetic rats as well as in response to treatment. For brevity, [18F]FDG liver data are not summarized.

Data optimization
The Levenberg-Marquardt algorithm was used to minimize the weighted residual sum of squares with the model optimized against the dynamic PET data. The weights used were proportional to the square root of the ratio of scan duration to tissue ROI activity.

Statistical analysis
All measures were expressed as mean ± SEM, except where noted otherwise. An unpaired t-test was used to determine the differences between ZDF and lean rats at baseline (i.e., 14 weeks) and between treated and untreated ZDF rats. A P-value less than 0.05 was considered significant.

Results
Plasma substrate levels and animal characteristics
Untreated ZDF rats were characteristically hyperglycemic, as shown by elevated plasma glucose and glycosylated hemoglobin levels compared to lean rats as shown in Table 1. Treatment with RGZ decreased HbA1c levels by 40% (P < 0.001), but we did not see improvement in fasting plasma glucose levels potentially due to the effects of anesthesia. In the untreated group, ZDF rats exhibited lower heart rates than lean rats. Plasma FFA levels were significantly higher (P < 0.05) in untreated ZDF rats compared to lean, while treated
ZDF rats showed no significant change in circulating FFAs. Treated rats gained more ($P < 0.001$) weight than untreated rats.

Brown adipose tissue  
**Baseline metabolic phenotype.** Untreated ZDF rats have significantly diminished ($P < 0.05$) transport of glucose compared to leans as evident in $K_1$ (Figure 2A). While the intrinsic glucose uptake rate constant was not significantly lower in ZDF rats, ZDF rats tended to have a lower GUR_int (Figure 2B). When taking into account the peripheral glucose levels, there were no significant differences in GUR_ext between lean and ZDF rats (Figure 2C). On the other hand, the uptake of [11C]Palmitate as estimated by $K_1$ was significantly lower in ZDF rats ($P < 0.001$; Figure 2A), while no significant differences in intrinsic measures of FAE were observed between the two groups (Figure 2B). Primarily due to higher peripheral levels of

### TABLE 1 Animal demographics and plasma substrate levels

|                | No Treatment | Treatment | ZDF+RGZ |
|----------------|--------------|-----------|---------|
| lean ZDF       |              |           |         |
| Weight (g)     | 306±11       | 322±18    | 344±21  |
| HR (bpm)       | 246±21       | 215±26a   | 198±15  |
| HbA1C (%)      | 3.33±0.12    | 7.69±0.56a| 7.38±0.73| 4.40±0.70b|
| Insulin (μU/mL)| 11.4±3.77    | 34.21±17.7a| 18±11  |
| Glucose (mM)   | 7.52±2.18    | 17.6±4.99a| 23±2.5  |
| FFA (μmol/L)   | 826±301      | 2320±1134a| 1920±891| 2029±642|

*P<0.05 Significantly different than Leans  
*P<0.05 Significantly different than untreated ZDF

![FIGURE 2](image-url)  
**FIGURE 2** Intrinsic and extrinsic measures of [18F]FDG and [11C]Palmitate uptake and metabolism in brown adipose tissue with treatment (left column) and without PPARc treatment (right column). Top row represents fractional transport of [18F]FDG and [11C]Palmitate; middle row depicts measures of [18F]FDG uptake rate constant and intrinsic FAE rate; and bottom row represents extrinsic measures of [18F]FDG uptake and FAE flux. Note the dual Y-axis with left axis representing glucose parameters and right axis representing FA parameters.
FA, extrinsic measures of FAE tended to be higher in ZDF rats, though the results did not reach significance (Figure 2C).

Effects of treatment. RGZ-treated rats exhibited significantly higher glucose and FA transport as shown in Figure 2D; however, there were no significant differences in intrinsic and extrinsic measures of glucose and FA uptake rate and esterification, although treatment with RGZ tended to reduce FAE (Figure 2E and F).

**Skeletal muscle substrate metabolism**

Baseline metabolic phenotype. At baseline, ZDF rats exhibited significantly lower transport of $[^{18}F]$FDG compared to lean rats (Figure 3A), consistent with the diabetic phenotype. However, due to higher peripheral concentration of glucose, the overall flux of glucose is higher in ZDF rats than lean rats (Figure 3C). Similarly due to the higher presence of FFA in the periphery, extrinsic measures of FA flux tended to be higher in ZDF rats than lean rats, though the results did not reach significance.

Effects of treatment. Treatment with PPARγ agonist tended to increase intrinsic measures of glucose uptake (Figure 3A) while diminishing intrinsic measures of FA uptake (Figure 3E). In particular, both FAE and FAE$_{UpR}$ were noticeably lower in RGZ-treated ZDF rats.

**Cardiac substrate metabolism**

Baseline metabolic phenotype. ZDF rats exhibit significantly lower intrinsic uptake of glucose, while, in parallel, ZDF rats exhibit significantly higher intrinsic rates of FAU (Figure 4A). When taking into account the peripheral levels of glucose and FFA, the higher concentration of glucose in ZDF rats ameliorates differences in GUR$_{ext}$ and exacerbates flux of FA into the myocardium (Figure 4B).

Effects of treatment. Overall, treatment with RGZ resulted in significantly higher ($P < 0.05$) uptake rate constant of glucose (Figure 4C) with significant reduction in both intrinsic ($P < 0.01$) and extrinsic ($P < 0.01$) measures of FAU (Figure 4D).

**Liver substrate metabolism**

No significant changes were observed in GUR$_{int}$ or GUR$_{ext}$ in the liver between lean and ZDF rats or following treatment (data not shown). Similarly, intrinsic measures of FA metabolism were not significantly different between the groups (Figure 5A). In contrast, ZDF rats exhibited significantly higher utilization of FA destined toward FAE storage, presumably as triglyceride (TG), due to the overall higher peripheral concentration of FFA (Figure 5B and C). Since RGZ did not cause a significant reduction in peripheral FA concentrations, extrinsic measures of FAU did not change following treatment. Interestingly, the flux of

![FIGURE 3](image)
Palmitate destined to FAE was significantly lower following RGZ therapy (Figure 5F).

Discussion

Systemic alterations in peripheral metabolism is one of the hallmarks of obesity and T2DM with current drugs and others in the pipeline targeting key pivots of substrate metabolism. The objective of this work, therefore, was to noninvasively quantify glucose and FA metabolism in tissues known to be affected in T2DM, in particular, BAT, skeletal muscle, heart, and the liver of lean and obese diabetic rats. In addition, we sought to assess the response of above-mentioned tissues to PPARγ therapy. Previous studies have characterized metabolic alterations independently in muscle (12,22,23) or heart (10,11). However, the simultaneous contribution of individual tissues has scarcely been reported. Guiducci et al. (24) recently characterized the contribution of blood flow and tissue clearance on extrinsic measures of FDG metabolism in multiple tissues. In contrast, in this work, we characterize both glucose and FA substrate metabolism in multiple tissues. Finally, as mentioned earlier, we make a distinction between intrinsic measures of substrate metabolism and extrinsic measures of flux, with the former being independent of peripheral substrate concentrations.

In BAT and muscle, we observed a reduction in glucose transport ($K_1$) in diabetic rats. The intrinsic glucose uptake rate constant, GUR$_{int}$, was significantly reduced in diabetic hearts but unchanged in BAT and muscle, suggesting an uncoupling of transport and phosphorylation processes in muscle and BAT. Interestingly, Williams et al. (25) reported no differences in $K_1$ or GUR$_{int}$ in skeletal muscle in a human PET study of obese and T2DM individuals using similar modeling methods. When examining the extrinsic uptake of glucose, GUR$_{ext}$, we observed no differences in GUR$_{ext}$ in BAT and heart between lean and diabetic rats due to the higher peripheral levels of glucose which masked the lower GUR$_{int}$ in diabetic rats. However, diabetic rats exhibited higher GUR$_{ext}$ in muscle than lean rats in agreement with previous reports (24,26,27). Thus, while the intrinsic cellular mechanisms in muscle, such as glucose transport as captured through the $K_1$ parameter, blunt glucose uptake, the higher peripheral concentration of glucose resulted in overall greater flux of glucose into tissue. In the liver, we did not observe differences in $[18F]FDG$ kinetics between lean and ZDF rats, most likely due to limitations in downstream metabolism of $[18F]FDG$ and due to high levels of glucose-6-phosphatase which result in clearance of $[18F]FDG$. Overall, our data indicate that ZDF rats have an innate deficiency in glucose transport in muscle, BAT, and heart, which is attributed to downregulation of GLUT transporters in the diabetic state (11,12,28).

RGZ improved insulin sensitivity more so in BAT than in muscle as seen by the significant increase in $K_1$, although glucose transport tended to be higher following therapy in muscle as well. Indeed, insulin has been shown to significantly increase glucose uptake in BAT (29). Moreover, tissue-specific knockout of insulin receptors in BAT has shown to promote a diabetic phenotype (30). We did not observe significant changes in GUR$_{int}$ and GUR$_{ext}$ in treated rats consistent with that found in humans (31). In addition, GUR$_{int}$ significantly improved in heart, while the increase in GUR$_{ext}$ trend did not reach significance. These results demonstrate the effects of PPARγ agonists on peripheral tissues and are in accord with previous reports on the efficacy of this drug class (28,32-34). One possible mechanism of thiazolidinediones (TZD) is the increased expression of insulin sensitive genes resulting in translocation of GLUT4 (10,13). GLUT4 translocation will lead to increased glucose disposal and a net decrease in peripheral glucose levels. We did not observe changes in glucose levels following therapy despite lower HbA1C levels, possibly due to the effects of anesthesia. However, our data clearly demonstrate therapeutic response of RGZ resulting in increased glucose transport ($K_1$), attributed to GLUT4 translocation as we have demonstrated previously (11).
Increased FA uptake has been implicated in the pathogenesis of the metabolic alterations that accompany T2DM and obesity (9). Our results show that ZDF rats have higher circulating FFA levels compared to lean. Interestingly, ZDF rats had decreased transport of $[^{11}C]$Palmitate compared to lean s both BAT and muscle. Previous studies by Bonen et al. and Luiken et al. (12,23) have shown that both increased FFAs and greater fatty acid transport (FAT)/cluster of differentiation 36 (CD36) protein content at the cell membrane account for greater FA transport in fat, muscle, and heart of ZDF rats. Conversely, Blaak et al. report impairment in FA uptake in obese and T2DM humans (35). Our data suggest that the amount of intracellular FA represented by the concentration in compartment 1 (Figure 1A) would be similar in lean and ZDF rats given the high plasma FFA levels. When considering the esterification of FAs, ZDF rats tended to have both greater intrinsic and extrinsic FAE in muscle, BAT, and heart, although no statistical significance could be reached for estimates in BAT and muscle. We assume that the increased FAE is not just attributed to elevated plasma FFA concentrations but also to an intracellular mechanism that partitions more FA toward storage. Thus, the actual metabolic impairment may be how FAs are handled once in the cell, which, in ZDF rats, seems to be a shift toward increased FAE. One theory is that tissues are operating beyond their oxidative capacity due to lowered number of mitochondria and/or higher flux and therefore store FFAs at a higher rate to compensate for the increased availability of lipids (22). The rise of IMTG levels, in turn, impairs muscle glucose uptake (4).

The effect of TZDs on FA metabolism has also been well studied in humans and animal models of diabetes. Coort and colleagues showed that RGZ increased FA uptake in adipose tissue but not in muscle or heart membrane vesicles in Zucker rats, due in part to greater FAT/CD36 and FATP1 concentration at the cell membrane (36). Palmitate oxidation in skeletal muscle was shown to increase in healthy rats but not ZDFs treated with RGZ (37,38). Our data clearly demonstrate a significant increase in FA transport in BAT with a nominal increase in muscle following RGZ. Both intrinsic and extrinsic measures of FAE tended to be lower in muscle and BAT. Similarly, overall FAU was significantly reduced in the heart. We have previously shown that RGZ inhibits FA oxidation in the heart which we attributed to blunt reduction in expression of genes encoding medium-chain acyl-CoA hydrolase (MCAD) and fatty acid transport protein 1 (FATP-I) (10). In the liver, we found that RGZ treatment caused significant ($P < 0.05$) reduction in FAE, in agreement with the reduced lipid storage with RGZ therapy in rats (39). Taken together, our data suggest that RGZ acts by reducing storage of exogenous FA in lipid pools, possibly by initiating a remodeling process that increases lipolysis of stored FA in non-adipose tissues and increases the efficiency of adipose tissue to store fat by increasing the number but decreasing the size of adipocytes (40).

In this study, we reported the metabolic dysfunction seen in the ZDF rat, an obese animal model of T2DM, and the effect of RGZ on improving insulin sensitivity and FAU in multiple affected tissues using preclinical PET and quantitative modeling. Glucose transport is impaired in the ZDF rat but is ameliorated by RGZ in heart, skeletal muscle, and brown fat. There is also a tendency toward increased esterification of FAs in these tissues, the severity of which was improved with RGZ therapy in a tissue-specific manner. Consequently, we have emphasized the importance of examining multiple tissues and using appropriate physiologic models to understand the biological mechanisms that arise in T2DM and during therapy. These developments highlight the potential to screen therapeutic interventions for diabetes, obesity, and metabolic diseases classified under the umbrella “metabolic syndrome” as well as to characterize the progression of diabetes and the interplay with cardiovascular disease.

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