Divergent effects of rosiglitazone on protein-mediated fatty acid uptake in adipose and in muscle tissues of Zucker rats

S. L. M. Coort,[1,*] W. A. Coumans,[*] A. Bonen,[†] G. J. van der Vusse,[§] J. F. C. Glatz,[*] and J. J. F. P. Luiken[*,]**

Departments of Molecular Genetics[*] and Physiology,[§] Cardiovascular Research Institute Maastricht, Maastricht University, Maastricht, The Netherlands; Department of Human Biology and Nutritional Sciences,[†] University of Guelph, Guelph, Ontario, Canada; and Department of Biochemical Physiology and Institute of Biomembranes,[**] Utrecht University, Utrecht, The Netherlands

Abstract **Thiazolidinediones (TZDs) increase tissue insulin sensitivity in diabetes. Here, we hypothesize that, in adipose tissue, skeletal muscle, and heart, alterations in protein-mediated FA uptake are involved in the effect of TZDs. As a model, we used obese Zucker rats, orally treated for 16 days with 5 mg rosiglitazone (Rgz)/kg body mass/day. In adipose tissue from Rgz-treated rats, FA uptake capacity increased by 2.0-fold, coinciding with increased total contents of fatty acid translocase (FAT/CD36; 2.3-fold) and fatty acid transport protein 1 (1.7-fold) but not of plasmalemmal fatty acid binding protein, whereas only the plasmalemmal content of FAT/CD36 was changed (increase of 1.7-fold). The increase in FA uptake capacity of adipose tissue was associated with a decline in plasma FA and triacylglycerols (TAGs), suggesting that Rgz treatment enhanced plasma FA extraction by adipocytes. In obese hearts, Rgz treatment had no effect on the FA transport system, yet the total TAG content decreased, suggesting enhanced insulin sensitivity. Also, in skeletal muscle, the FA transport system was not changed. However, the TAG content remained unaltered in skeletal muscle, which coincided with increased cytoplasmic adipose-type FABP content, suggesting that increased extramyocellular TAGs mask the decline of intracellular TAG in muscle. In conclusion, our study implicates FAT/CD36 in the mechanism by which Rgz increases tissue insulin sensitivity.—Coort, S. L. M., W. A. Coumans, A. Bonen, G. J. van der Vusse, J. F. C. Glatz, and J. J. F. P. Luiken. Divergent effects of rosiglitazone on protein-mediated fatty acid uptake in adipose and in muscle tissues of Zucker rats. *J. Lipid Res.* 2005. 46: 1295–1302.**

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Thiazolidinediones (TZDs) are a new class of insulin-sensitizing agents for the oral treatment of type 2 (non-insulin-dependent) diabetes. Several studies with insulin-resistant rodent models and type 2 diabetic patients have demonstrated that TZD treatment reduces both hyperinsulinemia and hyperlipidemia (1–5). Another important effect of TZDs is the normalization of insulin-stimulated whole-body glucose disposal, as reflected by an improved insulin action on skeletal muscle (2) and heart (4). TZDs are agonists of the peroxisome proliferator-activated receptor γ (PPARγ) (6, 7). PPARγ, a nuclear hormone receptor, is predominantly expressed in adipocytes but also, although at lower levels, in skeletal muscle and heart (8, 9). Together with the retinoid X receptor-α, PPARγ forms a heterodimer, and once activated this dimer modulates transcription patterns caused by the interaction with peroxisome proliferator response elements (PPREs) located in the promoter regions of many genes (6). One of the most potent members within the class of TZDs is rosiglitazone (Rgz), an agent that binds PPARγ with high affinity (6). Activation of PPARγ by TZDs regulates the transcription of genes involved in the differentiation of preadipocytes and the remodeling of adipose tissue, resulting in smaller, more insulin-sensitive adipocytes (3, 10). Moreover, TZDs have been proven to influence cellular long-chain FA uptake by changing the mRNA expression levels of several key players in the protein-mediated FA uptake process (1, 11).

Abbreviations: A-FABPc, adipose-type cytoplasmic fatty acid binding protein; FABPpm, plasmalemmal fatty acid binding protein; FAT/CD36, fatty acid translocase; FATP, fatty acid transport protein; PPARγ, peroxisome proliferator-activated receptor γ; PPRE, peroxisome proliferator response element; Rgz, rosiglitazone; TAG, triacylglycerol; TZD, thiazolidinedione.

[1] To whom correspondence should be addressed.
e-mail: s.coort@gen.unimaas.nl

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In most mammalian cells, the majority of FAs are taken up via a protein-mediated transport system (12). Three membrane-associated proteins have been identified as putative FA transport proteins: *i*) a 43 kDa plasmalemmal fatty acid binding protein (FABPpm), a homolog of mitochondrial aspartate aminotransferase; *ii*) a 63 kDa FA transport protein (FATP), a protein with very long-chain acyl-CoA synthetase activity (13); and *iii*) an 88 kDa highly glycosylated transmembrane protein, fatty acid translocase (FAT/CD36), the rat homolog of human CD36 (14). In muscle, FAT/CD36 is present not only at the plasma membrane but also in an intracellular storage pool (15). The membrane FA transport proteins are only involved in cellular FA transport when they are present at the plasma membrane. Intracellular FAT/CD36 translocates to the plasma membrane upon a variety of stimuli, such as by contractile stimuli that activate AMP kinase (16) and by insulin via the activation of phosphatidylinositol 3-kinase (17).

Studies on the insulin-sensitizing effect of Rgz treatment have been focused on the effects in adipose tissue or at the whole-body level. Therefore, in the present study, not only adipose tissue but also other metabolically important tissues, such as skeletal muscle and heart, were investigated. We hypothesize that alterations in both the capacity to take up FA and the total and plasmalemmal amounts of FA transport proteins in metabolically important tissues play a pivotal role in the insulin-sensitizing effect of TZDs. Evidence for a role of FAT/CD36 in Rgz's insulin-sensitizing effect has been studied in spontaneous hypertensive rats, which harbor a defective CD36 allele and in which Rgz failed to improve glucose tolerance and hypertriglyceridemia (18). Unfortunately, this particular study investigated only the whole-body effect of Rgz and not possible tissue-specific effects. A proper model in which to investigate tissue FA uptake capacity is giant membrane vesicles. These vesicles are oriented right-side-out and contain cytosolic FA binding proteins that serve as a FA sink, whereas FA metabolism has been entirely eliminated (19).

In the present study, we advance the understanding of the in vivo action mechanism by which Rgz improves insulin sensitivity at three levels: the effects of Rgz *i*) on FA transport capacity in separate tissues (i.e., adipose tissue, skeletal muscle, and heart); *ii*) on protein levels of FA transport proteins (i.e., FAT/CD36, FABPpm, and FATP1), whereas other studies measured only mRNA levels (20, 21); and *iii*) on the plasmalemmal localization of these FA transport proteins. The latter provides a superior indication of the functionality of the FA transport proteins, because changes in mRNA levels are not necessarily accompanied by alterations in protein content and function of the FA transport proteins. In addition, total tissue triacylglycerol (TAG) content was determined in skeletal muscle and heart obtained from control and Rgz-treated obese animals. To discriminate between intramyocellular and extramyocellular lipids, we determined, in skeletal muscle and heart, adipose-type cytoplasmic fatty acid binding protein (A-FABPc), which is a marker of differentiated adipocytes (22). The present study indicates that in obese Zucker rats an increased plasmalemmal protein content of FAT/CD36 in adipose tissue is implicated in the mechanism by which TZDs redirect the FA flux from muscle cells toward adipose tissue, causing plasma FA and TAG concentrations and intramyocellular TAG content to decline.

EXPERIMENTAL PROCEDURES

Animals

Eleven week old female obese Zucker rats obtained from the Harlan Laboratory (Horst, The Netherlands) were individually housed on a 12 h light/12 h dark cycle and divided into two groups of eight animals each. The control group was fed a standard rat chow, and the Rgz-treated group received standard rat chow supplemented with 5 mg of Rgz (Avandia®; GlaxoSmithKline) per kilogram of body mass per day for 16 days. Body mass and food intake were monitored throughout the entire treatment period. The Experimental Animal Committee of Maastricht University gave approval for all experiments involving animals. Before tissue harvesting, rats were anesthetized with Nembutal® (sodium phenobarbital) injected intraperitoneally. We collected epididymal fat pads, the hindlimb muscle from the left leg, consisting of gastrocnemius and plantarius muscles, and the heart, thereby removing all the visible fat pads. After harvesting, tissues were separated into two parts; one part was used for the preparation of giant membrane vesicles, and the other part was immediately frozen in liquid nitrogen.

In blood plasma, collected directly after the rats were killed, glucose (hexokinase method; Roche, Basel, Switzerland), fatty acids (Wako NEFA C test kit; Wako Chemicals, Neuss, Germany), and TAGs (glycerol kinase-lipase method; Boehringer Mannheim, Mannheim, Germany) were measured with a COBAS BIO analyzer (COBAS FARA semiautomatic analyzer; Roche). The distribution of cholesterol in the three lipoprotein fractions (i.e., VLDL, LDL, and HDL) in plasma was determined with an AKTA-Basic chromatography system with a Superose 6PC.

Materials

[9,10-^{3}H]palmitate and [^{14}C]mannitol were obtained from American Radiolabeled Chemicals, Inc. (St. Louis, MO). Collagenase type II was obtained from Worthington Biochemical Co. (Lakewood, NJ). Collagenase IIa, collagenase VII, and BSA fraction V (fatty acid free) were obtained from Sigma-Aldrich (St. Louis, MO).

Isolation of giant membrane vesicles

Giant membrane vesicles from adipose tissue, skeletal muscle, and heart derived from control and Rgz-treated obese Zucker rats were prepared as described previously (20, 23). Briefly, tissues were cut into thin layers (1–3 mm thick) and incubated for 1 h at 34°C in 140 mmol/l KCl, 10 mmol/l MOPS (pH 7.4), aprotinin (10 mg/ml), and collagenase under continuous shaking. Collagenase type VII (150 U/ml) was used for skeletal muscle, collagenase type II (0.3%, w/v) for heart, and collagenase IIa (0.05%, w/v) for adipose tissue. At the end of the incubation, the supernatant fractions were collected and the remaining tissue was washed with KCl/MOPS and 10 mmol/l EDTA, which resulted in a second supernatant fraction. Both supernatant fractions were pooled, and Percoll and aprotinin were added to final concentrations of 16% (w/v) and 10 mg/ml, respectively. The resulting suspension was placed at the bottom of a density gradient consisting of a 3 ml middle layer of 4% Nycodenz (w/v) and a 1 ml KCl/MOPS upper layer. This sample was centrifuged at 60 *g* for 45 min at room temperature. Subsequently, the giant membrane vesicles were harvested from the interface of the upper and middle layers, diluted in KCl/MOPS, and recentrifuged at

900 *g* for 10 min. In the case of skeletal muscle, the pellet was resuspended in KCl/MOPS to a protein concentration of 2–3 mg/ml; with the other tissues, the pellet was resuspended to a protein concentration of 0.4–0.8 mg/ml.

[9,10-^{3}H]palmitate uptake by giant membrane vesicles

Vesicular [9,10-^{3}H]palmitate uptake was measured as described previously (20, 23). Briefly, 40 μl of 0.1% BSA in KCl/MOPS containing unlabeled (15 μmol/l) and radiolabeled [9,10-^{3}H]palmitate (0.3 μCi) and [^{14}C]mannitol (0.06 μCi) was added to a 40 μl giant membrane vesicle suspension. Mannitol is used to correct for nonspecific binding of palmitate. The incubation was carried out for 15 s. Vesicular [9,10-^{3}H]palmitate uptake was terminated by the addition of 1.4 ml of ice-cold KCl/MOPS, 2.5 mmol/l $HgCl_2$ and 0.1% BSA. The suspension was then quickly centrifuged at 12,000 *g* for 1 min. The supernatant fraction was discarded, and radioactivity was determined in the tip of the tube. Nonspecific uptake was measured by adding the stop solution before addition of the radiolabeled palmitate solution.

Tissue triacylglycerol content

Total TAG content was determined in whole homogenates of skeletal muscle and heart derived from control and Rgz-treated obese Zucker rats. Lipids were extracted and separated by high-performance thin-layer chromatography as described previously (24). Quantification was performed by scanning high-performance thin-layer chromatography plates and by integrating the density areas against an internal standard using Quantity One software (Bio-Rad Laboratories, Hercules, CA).

FAT/CD36, FABPpm, and FATP1 protein determination in homogenates and giant membrane vesicles

FAT/CD36, FABPpm, and FATP1 protein contents were determined in homogenates and giant membrane vesicles derived from heart, skeletal muscle, and adipose tissue from control and Rgz-treated obese Zucker rats. Aliquots of these homogenates (10 μg) and giant membrane vesicles (5 μg) were separated by 12% SDS-PAGE at 200 V for 55 min. Proteins were then transferred to a Trans-Blot® pure nitrocellular membrane (Bio-Rad Laboratories) at 100 V for 75 min. After blotting, the membrane was blocked for 1 h at room temperature with TBS-T [20 mM Tris base, 137 mM NaCl, 0.1 M HCl (pH 7.5), and 0.1% Tween 20] containing 7.5% BSA for FAT/CD36 and 5% nonfat dry milk for FABPpm and FATP1. A monoclonal antibody (MO25; 1:20,000) directed against human CD36, a rabbit monoclonal anti-FABPpm antiserum (1:3,000), and a rabbit polyclonal IgG antibody (1:1,000) directed against FATP1 (M-100; Santa Cruz Biotechnology, Santa Cruz, CA) were used to detect FAT/CD36, FABPpm, and FATP1, respectively. The primary antibody was incubated overnight at 4°C and then washed five times for 5 min each with TBS-T. After washing, the membrane was incubated for 1 h at room temperature with the secondary antibody, rabbit anti-mouse polyclonal HRP (1:20,000) for FAT/CD36 and swine anti-rabbit polyclonal HRP (1:3,000) for FABPpm and FATP1 (DAKO, Glostrup, Denmark). Thereafter, the membrane was washed five times for 5 min each with TBS-T and two times for 5 min each with TBS. Protein bands were visualized using ECL, and immunoblot intensities were analyzed by densitometry using the computer program Scion Image. The antibody directed against FATP1 appeared not entirely specific, and a few additional proteins bands were visible after using ECL. By using a molecular mass protein marker, we identified the 63 kDa protein band (FATP1).

Assay of A-FABPc

The content of A-FABPc was measured in adipose tissue, skeletal muscle, and heart homogenates by Western blotting using human anti-A-FABPc (HyCult Biotechnology BV, Uden, The Netherlands).

Statistical analysis

All data are presented as means ± SEM. Differences between control and Rgz-treated obese Zucker rats were tested by ANOVA and appropriate post hoc analysis between groups. $P < 0.05$ indicates statistical significance.

RESULTS

Characteristics of control and Rgz-treated obese Zucker rats

Obese Zucker rats treated with Rgz gained significantly more body mass and had a higher food intake throughout the treatment period than their nontreated obese controls (**Table 1**). However, the calculated metabolic efficiency (i.e., the gain in body mass divided by the amount of food consumed after 16 days of treatment) was significantly higher in the Rgz group compared with the control group (Table 1). Heart mass was significantly higher in the Rgz-treated animals, but after correction for body mass there was no difference. In addition, plasma glucose, TAG, and FA concentrations were reduced significantly by 35, 53, and 56%, respectively, in the Rgz-treated group compared with their controls ($P < 0.05$; Table 1). However, in response to the Rgz treatment, plasma cholesterol levels increased significantly by 1.7-fold in obese Zucker rats ($P < 0.05$; Table 1). This increased plasma cholesterol was equally distributed among the three different lipoprotein fractions (i.e., VLDL, LDL, and HDL) (data not shown). These effects of Rgz are characteristic of the treatment of insulin resistance with PPARγ agonists in rodent and human studies (2, 3, 25–27).

Whether the observed decline of plasma FA levels in obese Zucker rats upon Rgz treatment results in reduced FA availability for metabolically important tissues can be deduced from a comparison between the estimation of the FA concentration and the apparent K_m of these tissues for FA. The driving force of cellular FA uptake is the non-protein-

TABLE 1. Characteristics of control and Rgz-treated obese Zucker rats

Parameter	Control	Rgz
Body and organ mass		
Body mass at start (g)	410 ± 12	404 ± 13
Body mass gain over 16 days (g)	45.6 ± 15.6	89.3 ± 11.4[a]
Food intake over 16 days (g)	374 ± 7	486 ± 11[a]
Metabolic efficiency (%)[b]	10.9 ± 2.9	17.6 ± 1.7[a]
Heart mass (g)	1.07 ± 0.10	1.17 ± 0.10[a]
Heart/body mass (×10^3)	0.24 ± 0.02	0.24 ± 0.02
Hindlimb mass (g)	2.60 ± 0.16	2.44 ± 0.17
Hindlimb/body mass (×10^3)	0.57 ± 0.02	0.50 ± 0.04
Plasma metabolites (mmol/l)		
Glucose	17.5 ± 1.3	11.3 ± 1.6[a]
Triacylglycerols	6.30 ± 1.08	2.49 ± 0.57[a]
Fatty acids	0.39 ± 0.06	0.17 ± 0.03[a]
Cholesterol	1.9 ± 0.2	3.3 ± 0.1[a]

Rgz, rosiglitazone. Data are presented as means ± SEM (n = 8).
[a] Significantly different from obese control rats.
[b] Metabolic efficiency = (total body mass gain/total amount of food consumed) × 100%.

bound FA concentration, which is solely a function of the ratio of total FA to albumin concentrations (28). Corresponding with a decline of the total FA plasma levels from 0.4 to 0.2 mmol/l, the plasma non-protein-bound FA concentration decreases from 3.8 to 1.7 nmol/l (calculated for a mixture of palmitate and oleate, the two major FA species in plasma) (28). Because these FA concentrations are well below the apparent K_m of 9.7 nmol/l for the FA transport system in myocytes (23), a decrease in plasma FAs will result in a proportionally diminished cellular FA uptake rate, assuming that the albumin concentrations did not change.

Cellular fatty acid uptake capacity

Giant membrane vesicles were used to investigate the capacity for FA uptake across the plasmalemma of adipose tissue, skeletal muscle, and heart. The giant membrane vesicles allow the measurement of FA uptake without the confounding effects of FA metabolism (29). These vesicles are oriented right-side-out and contain abundant quantities of FABPc, which serves as a FA sink (19). To be able to compare the FA uptake capacity among different tissues, the concentration of non-protein-bound [^{3}H]palmitate was equal in each experiment and set at 5.1 nM. This concentration is below the apparent K_m (9.7 nM) for the transport system for myocytes (23) and likely also for that of adipocytes (21), so that in vivo FA uptake rates are a linear function of the (non-protein-bound) FA concentration, as explained above. After 16 days of Rgz treatment, the capacity to take up FAs was increased significantly (i.e., by 2.0-fold) in giant vesicles derived from adipose tissue (**Fig. 1A**). In contrast, Rgz treatment had no effect on FA uptake capacities in skeletal muscle and heart (Fig. 1B, C).

Total and plasmalemmal FAT/CD36, FABPpm, and FATP1 protein contents

In control and Rgz-treated obese Zucker rats, the total protein contents of FAT/CD36, FABPpm, and FATP1 were measured in homogenates from adipose tissue, skeletal muscle, and heart (**Fig. 2A, C, E**), whereas plasmalemmal FAT/CD36, FABPpm, and FATP1 protein contents were determined in the giant membrane vesicle preparations (Fig. 2B, D, F). In skeletal muscle and heart, neither total nor plasmalemmal amounts of FAT/CD36 and FABPpm were altered by Rgz treatment of obese Zucker rats (Fig. 2). In contrast, Rgz treatment increased the total amount of FAT/CD36 in adipose tissue by 2.3-fold ($P < 0.05$), with a concomitant increase in adipocyte plasmalemmal FAT/CD36 by 1.7-fold (Fig. 2A, B). The total and plasmalemmal protein levels of FABPpm in adipose tissue were not altered by Rgz treatment (Fig. 2C, D). However, in adipose tissue, the total amount of FATP1 increased by 1.7-fold after Rgz treatment, whereas in skeletal muscle and heart, FATP1 remained unaltered (Fig. 2E). In giant membrane vesicles from adipose tissue, skeletal muscle, and heart, the content of FATP1 was below the detection level (Fig. 2F). In a previous study using a different antibody, FATP1 was also barely detectable in giant membrane vesicles from skeletal muscle and heart (23).

Total tissue TAG and A-FABPc contents

The total tissue TAG and A-FABPc contents were measured in skeletal muscle and heart homogenates obtained from control and Rgz-treated obese Zucker rats. Rgz treatment significantly decreased the total tissue amount of TAGs in heart by 36%. In skeletal muscle, total tissue TAG content was not significantly different between control and Rgz-treated obese animals (**Fig. 3A**). In adipose tissue and skeletal muscle from obese Zucker rats, Rgz administration significantly increased A-FABPc protein contents by 1.4-fold and 1.2-fold, respectively ($P < 0.05$; Fig. 3B). A-FABPc was almost undetectable in heart homogenates from both control and Rgz-treated obese rats (Fig. 3B).

DISCUSSION

The present study implicates FAT/CD36 in the insulin-sensitizing action of Rgz in obese Zucker rats. Total and plasmalemmal FAT/CD36 protein content increased in adipose tissue, coinciding with an increased FA uptake transport capacity. Plasmalemmal protein contents of both FABPpm and FATP1 were unaltered in adipose tissue of

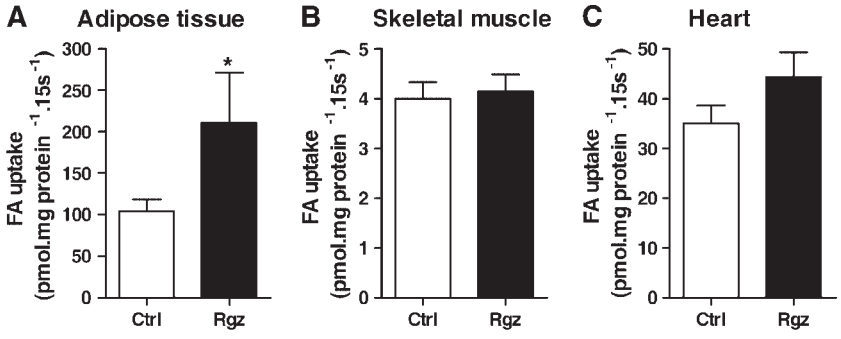

Fig. 1. [^{3}H]palmitate uptake capacity in giant membrane vesicles derived from metabolically important tissues of obese Zucker rats. [^{3}H]palmitate (FA) uptake in 15 s was measured in giant membrane vesicles from adipose tissue (A), skeletal muscle (B), and heart (C) derived from control (Ctrl; open bars) or rosiglitazone (Rgz)-treated (closed bars) obese Zucker rats. Data are presented as means ± SEM (n = 8). * Significantly different from control obese animals ($P < 0.05$).

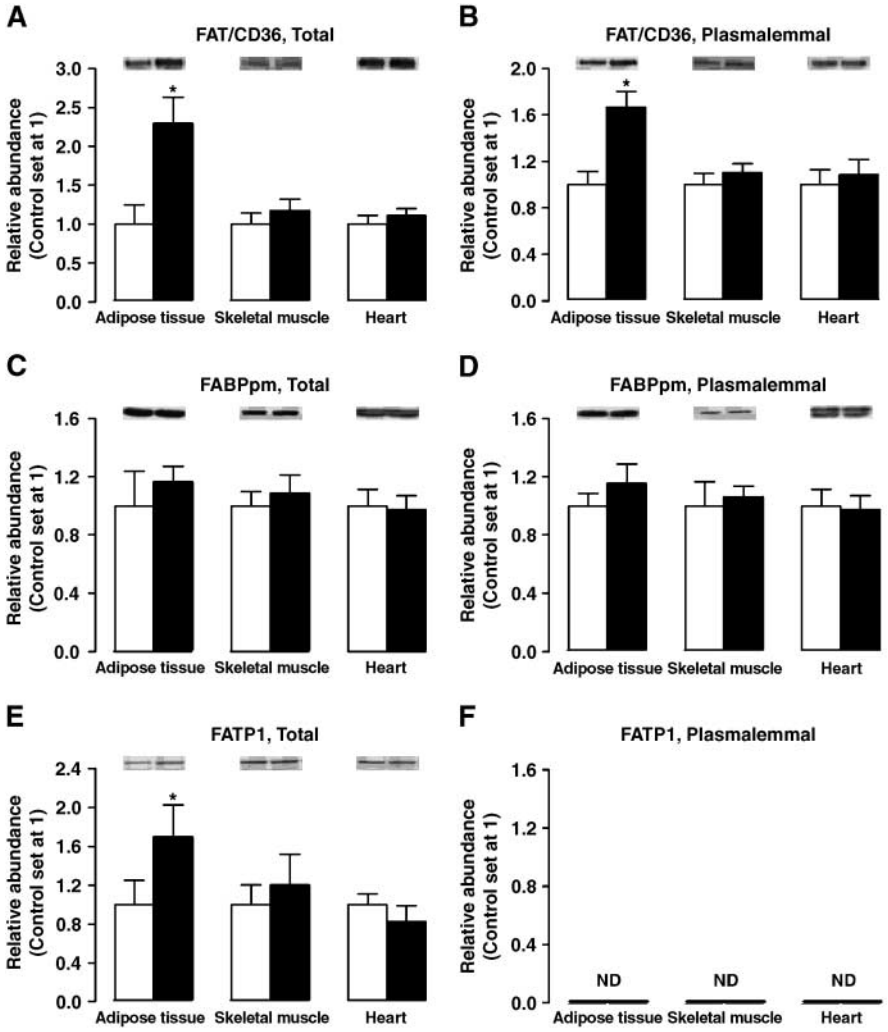

Fig. 2. Total and plasmalemmal protein contents of fatty acid translocase (FAT/CD36), plasmalemmal fatty acid binding protein (FABPpm), and fatty acid transport protein 1 (FATP1) in adipose tissue, skeletal muscle, and heart. Total protein contents of FAT/CD36, FABPpm, and FATP1 were measured in 10 μg of total homogenates (A, C, and E), and plasmalemmal protein contents were measured using 5 μg of giant membrane vesicle preparation (B, D, and F), both derived from control (open bars) or Rgz-treated (closed bars) obese Zucker rats. Representative Western blots are given per tissue (adipose tissue, skeletal muscle, or heart) and per fraction (total or plasmalemmal). The Western blot intensities of the different experiments were analyzed by densitometry using the computer program Scion Image. Per group, either control obese rats or Rgz-treated obese rats, the mean value of the densities was calculated and the mean value of the control rats was set at 1. Thus, data are presented as means ± SEM, corresponding to control values (n = 8). * Significantly different from control obese animals ($P < 0.05$). ND, not detectable.

Rgz-treated obese rats, suggesting no functional role for these putative FA transport proteins in the increased FA uptake capacity. Rgz treatment decreased total cardiac TAG content, whereas in skeletal muscle, the total TAG content remained unaltered. Interestingly, we demonstrated that in skeletal muscle of Rgz-treated rats, the A-FABPc content increased, indicating substantial extramyocellular deposition of differentiated adipocytes.

Effect of Rgz treatment on cellular long-chain fatty acid uptake capacity

Alterations in cellular FA uptake have been proposed to play a prominent role in the insulin-sensitizing effect of TZDs in obesity and type 2 diabetes. Recently, we demonstrated that the FA uptake capacity is increased in adipose tissue, heart, and skeletal muscle from insulin-resistant obese Zucker rats compared with lean control rats (20). However, limited data are available on Rgz's effect on the cellular FA uptake capacity in various metabolically important tissues from obese rats. Therefore, in the present study, we determined the FA uptake capacity in adipose tissue, skeletal muscle, and heart using giant membrane vesicles as a model. We are the first to demonstrate that the FA uptake capacity increases significantly by 2.0-fold in adipose tissue from Rgz-treated obese rats compared with control obese rats. The present findings provide a mechanistic insight for findings from Oakes and coworkers (3), who calculated, using an in vivo tracer method, that darglitazone in-

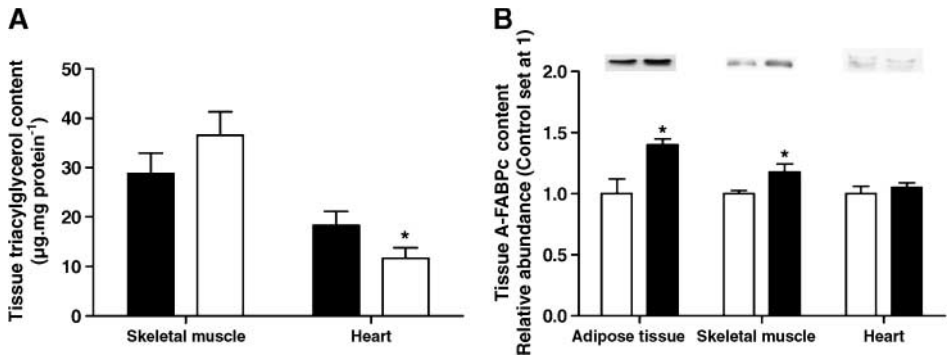

Fig. 3. Total tissue triacylglycerol (TAG) content in skeletal muscle and heart (A) and adipose-type cytoplasmic fatty acid binding protein (A-FABPc) content in adipose tissue, skeletal muscle, and heart (B) from control (open bars) and Rgz-treated (closed bars) obese Zucker rats. Tissue TAG content was measured using high-performance thin-layer chromatography and is presented as means ± SEM (n = 8). A-FABPc content was determined by Western blotting; representative blots are given and quantitated relative to corresponding control values, which were set at 1 (n = 4). * Significantly different from control obese animals ($P < 0.05$).

creases by 2.3-fold the deposition of plasma FA into adipose tissue from obese Zucker rats.

In contrast to adipose tissue, in skeletal muscle and heart the FA uptake capacity was unaffected by Rgz treatment in obese rats. However, we calculated that by reducing the plasma FA and TAG levels, the availability of these substrates for nonadipose tissue, such as skeletal muscle and heart, declines proportionally (see Results). Thus, in contrast to adipose tissue, both skeletal muscle and heart do not compensate for the decline in plasma FA availability by increasing their FA uptake capacity.

Changes in membrane fatty acid transport protein levels after Rgz treatment

In the present study, we showed that Rgz treatment had differential effects on the FA uptake capacity in adipose and muscle tissues from obese rats. It is well documented that plasmalemmal FA uptake is mediated predominantly by FA transport proteins (12), and several candidate proteins have been identified to play a role in this transport system: FAT/CD36, FABPpm, and FATP (14). Here, we demonstrated that in adipose tissue, total FAT/CD36 and FATP1 protein contents were increased significantly in Rgz-treated obese Zucker rats, whereas the total FABPpm protein content was unaltered. This is in agreement with the observation that both FAT/CD36 and FATP1 have a PPRE (30). It is unknown whether a PPRE is present in the upstream regions of the FABPpm gene. The fact that Rgz has an effect on FAT/CD36 and FATP1 and not on FABPpm suggests that such a PPRE is lacking in the FABPpm gene. Others already showed that mRNA levels of FAT/CD36 and FATP1 are upregulated in adipose tissue derived from TZD-treated insulin-resistant rodents (1, 11). Apparently, changes in mRNA levels of FAT/CD36 and FATP1 are in accordance with their respective total protein levels.

In addition to the amount of membrane-associated FA transport proteins, their subcellular localization is important for their function (15). In earlier studies, we demonstrated that FAT/CD36 and FABPpm are present both at the plasma membrane and in intracellular storage pools (15, 31). Moreover, we found a positive correlation between the amount of FAT/CD36 residing at the plasma membrane and the rate of cellular FA uptake (23).

In the present study, we demonstrated that upon Rgz treatment the plasmalemmal content of FAT/CD36 was increased by 1.7-fold, closely matching the increase of 2.3-fold in its total expression and the 2.0-fold induction of the plasmalemmal capacity to take up FA. Thus, the additional FAT/CD36 expressed is distributed equally between intracellular storage pools and the plasma membrane. In contrast to FAT/CD36, both the total and plasmalemmal amounts of FABPpm did not change. Although the content of FATP1 in adipose tissue increased upon Rgz treatment, FATP1 was undetectable in giant membrane vesicles from adipose tissue and in vesicles from skeletal muscle and heart. This latter finding suggests that FATP1 plays quantitatively no prominent role in the transmembrane transport of FAs. Rather, FATP1 may function in the intracellular trapping of FAs, presumably by its acyl-CoA synthetase activity (32). The Rgz-induced 1.4-fold increase of A-FABPc in adipose tissue relates to the presence of a PPRE also in the gene of this protein (14, 30). However, because FABPc's have a permissive and no regulatory role in cellular FA uptake (14), this increase will hardly influence the rate of FA uptake into adipose tissue. Therefore, these combined observations indicate that the increase in FAT/CD36 expression in adipose tissue is pivotal in explaining the increase in cellular FA uptake.

In contrast to adipose tissue, Rgz treatment had no effect on total or plasmalemmal protein contents of FAT/CD36, FABPpm, and FATP1 in skeletal muscle and heart (Fig. 2). These observations are in agreement with the unaltered FA uptake capacity in skeletal muscle and heart from Rgz-treated obese rats (this study) and with the low expression of PPARγ in these tissues (33).

Effect of Rgz on total TAG contents and A-FABPc protein levels in skeletal muscle and heart

It is well documented that the accumulation of intramyocellular TAGs positively correlates with the development

of tissue insulin resistance (34, 35). Recently, we demonstrated that the total TAG content was 3-fold higher in hearts of obese Zucker rats than in their age-matched controls (36). The present study showed that after Rgz treatment, the total cardiac TAG content of obese Zucker rats declined significantly, suggesting that the heart becomes more insulin sensitive. A reduction in intramyocellular TAG is not only beneficial for the tissue sensitivity to insulin but also for the functioning of the heart. Young and coworkers (37) demonstrated in obese rats a positive correlation between contractile dysfunctioning and an increase in intramyocellular lipids. Moreover, an enhanced protection of the heart against ischemic injury after TZD treatment was demonstrated by Sidell and coworkers (27).

In contrast to heart, Rgz treatment had no effect on the total TAG content in skeletal muscle from obese rats. Puzzling findings were presented by Muurling et al. (4), who showed an increased amount of TAG in skeletal muscle from Rgz-treated ob/ob mice, whereas the whole-body insulin sensitivity improved. In our study and the one by Muurling and coworkers, total TAG content was measured in total tissue homogenates, making it impossible to discriminate between intramyocellular and extramyocellular TAG. Therefore, the amount of A-FABPc, an established marker of differentiated adipocytes (22), was measured in tissue homogenates and demonstrated to be increased by 1.4-fold and 1.2-fold in adipose tissue and skeletal muscle, respectively, after Rgz treatment, whereas in heart, A-FABPc was almost undetectable in Rgz-treated obese rats. In type 2 diabetic patients treated for 3 months with TZDs, it was demonstrated that the extramyocellular TAG content in skeletal muscle increases by 1.4-fold (38). A-FABPc is expressed in differentiated adipocytes and not in skeletal muscle; thus, adipose tissue deposition between muscle fibers increases. Because PPARγ activation elicits the differentiation of adipocytes (10), we believe that the increase in muscular adipocytes is attributable to the differentiation of existing preadipocytes. Accordingly, an increased deposition of adipocytes between skeletal muscle fibers could mask a possible decline of intramyocellular TAG in skeletal muscle cells after Rgz treatment.

Concluding remarks

One of the hallmarks of obesity and type 2 diabetes is tissue insulin resistance (39). It is well described that treatment of type 2 diabetes with TZDs improves insulin sensitivity (6, 25), exemplified by increased cellular glucose uptake in both skeletal muscle and heart from TZD-treated obese Zucker rats (5, 27). Here, we show that an increased plasmalemmal protein content of FAT/CD36 in adipose tissue plays a pivotal role in the insulin-sensitizing effect of Rgz treatment in obese Zucker rats. As a result of Rgz treatment, adipose tissue extracts FA from the circulation, thereby reducing the FA supply to nonadipose tissue such as skeletal muscle and heart. The reduced FA availability reduces the myocytal FA uptake rate and limits intramyocellular TAG accumulation, leading to increased muscle insulin sensitivity. Thus, the improvement of muscle insulin sensitivity can be considered secondary to the direct effect of Rgz on FAT/CD36-mediated FA uptake by adipose tissue.

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