

# Intramyocellular Lipid and Insulin Resistance

## A Longitudinal In Vivo $^1\text{H}$ -Spectroscopic Study in Zucker Diabetic Fatty Rats

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**Insulin resistance plays an important role in the pathogenesis of human type 2 diabetes. In humans, a negative correlation between insulin sensitivity and intramyocellular lipid (IMCL) content has been shown; thus, IMCL becomes a marker for insulin resistance. Recently, magnetic resonance spectroscopy (MRS) has been established as a dependable method for selective detection and quantification of IMCL in humans. To validate the interrelation between insulin sensitivity and IMCL in an animal model of type 2 diabetes, we established volume selective  $^1\text{H}$ -MRS at 7 Tesla to noninvasively assess IMCL in the rat. In male obese Zucker Diabetic Fatty rats and their lean littermates, IMCL levels were determined repeatedly over 4 months, and insulin sensitivity was measured by the euglycemic-hyperinsulinemic clamp method at 6–7 and at 22–24 weeks of age. A distinct relation between IMCL and insulin sensitivity was demonstrated as well as age dependence for both parameters. Rosiglitazone treatment caused a clear reduction of IMCL and hepatic fat despite increased body weight, and a marked improvement of insulin sensitivity. Thus, the insulin sensitizing properties of rosiglitazone were consistent with a redistribution of lipids from nonadipocytic (skeletal muscle, liver) back into fat tissue. *Diabetes* 52:138–144, 2003**

**I**nsulin resistance (IR) is a common feature of the metabolic syndrome and an important factor in the cause of type 2 diabetes. There is abundant evidence that increased levels of plasma lipids, predominantly free fatty acids (FFAs) and triglycerides, are causally involved in IR (1). In the 1960s, Randle et al. (2) introduced the idea that FFAs interfere with insulin action in peripheral tissues. His group suggested a substrate competition

between plasma glucose and FFAs as fuel for energy production, the “glucose fatty-acid cycle.” In humans, lipid infusion plus heparin, which acutely raises plasma FFA levels, inhibits insulin-stimulated glucose uptake in muscle, a finding consistent with increased peripheral IR. However, in a temporal sequence, this FFA-mediated impairment of insulin-stimulated glucose utilization developed with a delay of  $\sim 3$  h after starting the lipid infusion (3,4) and was accompanied by a fall rather than a rise in the muscle glucose-6-phosphate concentration (5) as expected by the original Randle hypothesis (6,7). In animal models, an increased IR was also observed after lipid infusion or high-fat feeding (8,9), which was accompanied by a rise of the triglyceride content in liver and skeletal muscle (10,11).

On the basis of these findings and of the well-known fact that peripheral insulin sensitivity (IS) depends predominantly on glucose utilization in muscle tissue, recently not only the higher levels of circulating lipids but also the role of lipids accumulating within muscle cells received increasing attention. Currently, the ectopic deposition of fat in nonadipose tissue is considered an important aspect in the development of IR (1,7,12). Muscle triglycerides themselves do not seem to interfere directly with insulin action in the myocytes but rather to act through some other fatty acid-derived metabolite to impair insulin signaling. Long-chain fatty acyl-CoAs have been suggested as possible candidates (13–15).

In muscle tissue, lipids are stored either as interstitial adipocyte triglycerides (termed extramyocellular lipids [EMCLs]) or as intramyocellular lipids (IMCLs), accumulating as droplets in the cytoplasm of muscle cells. Whereas EMCL is metabolically relatively inert, IMCL stores are built up, mobilized, and used within several hours (8,16–18). Recent data indicate a strong correlation between accumulation of IMCLs and IS in humans, not only in diabetic patients but also in glucose-tolerant and -intolerant individuals with or without obesity (19–21). Determination of muscle triglycerides was classically only possible by invasive techniques (22–24). Recently, a non-invasive method, volume-localized  $^1\text{H}$  magnetic resonance spectroscopy (MRS), was established (25–27). This method offers the unique ability to distinguish between IMCL and EMCL in vivo and was proved to provide reliable quantification of IMCL. Noninvasive quantification of muscle lipids with MRS has established that IMCL is a reliable marker for IS in humans (21,28–32).

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EMCL, extramyocellular lipid; FFA, free fatty acid; GIR, glucose infusion rate; IMCL, intramyocellular lipid; IR, insulin resistance; IS, insulin sensitivity; MRS, magnetic resonance spectroscopy; PPAR- $\gamma$ , peroxisome proliferator-activated receptor- $\gamma$ ; RGZ, rosiglitazone; tCR, total creatine; TE, echo time; TR, repetition time; TZD, thiazolidinedione.

The aim of this study was severalfold. First, we wanted to monitor for the first time noninvasively and repeatedly the levels of IMCL in lean and obese male ZDF rats over a 4-month period. Our intention was to validate the relation between IMCL levels and IR in this animal disease model of spontaneous progression to type 2 diabetes. Second, we wanted to assess the effect of the insulin sensitizer rosiglitazone (RGZ), a peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ ) agonist (33–35), on IS and IMCL content.

## RESEARCH DESIGN AND METHODS

**Animals.** Male obese ZDF rats (ZDF/Gmi, *fa/fa*) and their lean littermates (ZDF/Gmi, *+/+*) were obtained from Genetic Models (Genetic Models, Indianapolis, IN). Animals were housed in pairs at 20°C and on a 12-h light-dark cycle with ad libitum access to water and standard rat diet (Altromin, Germany) containing 4% fat, 64% carbohydrate, and 19% protein. Twenty-nine obese and 21 lean ZDF rats were divided into three different groups for three study protocols A, B, and C.

**Experimental protocols.** All experimental procedures were conducted according to the German Animal Protection Law.

**Protocol A.** In eight obese and eight lean ZDF rats, skeletal muscle IMCL was monitored by MRS longitudinally over 4 months at the age of 6, 8, 10, 14, 18, and 22 weeks.

**Protocol B.** IS of five obese and five lean ZDF rats at the age of 6–7 weeks was determined in an euglycemic-hyperinsulinemic glucose clamp study.

**Protocol C.** Eight obese ZDF rats were treated with RGZ at a dose of 3 mg  $\cdot$  kg $^{-1}$   $\cdot$  day $^{-1}$  by food admixture starting at the age of 6 weeks and continuing for 4 months. Another eight obese and eight lean ZDF rats served as control groups. Food, water intake, and body weight were recorded weekly. Blood glucose, FFAs, triglycerides, cholesterol, and insulin were determined several times throughout the study as indicated in the figures. Liver fat content was determined at 17 weeks of age using volume selective MRS. IMCL content was monitored by MRS when the animals were 16 and 22 weeks old. At the age of 22–24 weeks, the IS of all rats was assessed in a glucose clamp study.

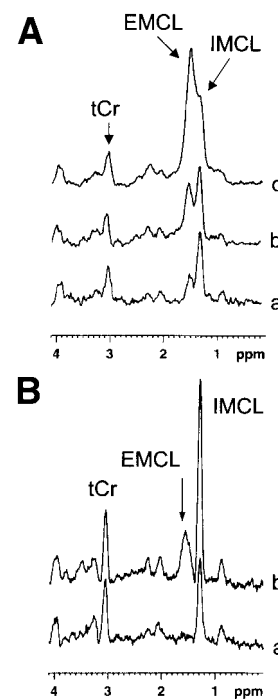
**MRS (protocols A and C).** In vivo MRS studies were performed on a 7 Tesla Biospec system (Bruker BioSpin, Ettlingen, Germany) using a resonator for excitation and an actively decoupled surface coil for signal detection. Rats were anesthetized with 2–3 vol% isoflurane and 1:2 O $_2$ :N $_2$ O, and their temperature was kept at 37.5°C. The animals were fixed in a nonmagnetic device allowing for accurate alignment of their hindleg on top of the surface coil. Voxels of  $\sim$ 8 mm $^3$  size were located in M. soleus and in M. tibialis anterior, avoiding vascular structures and gross adipose tissue deposits. Volume-localized  $^1$ H-MR spectra (PRESS sequence, echo time [TE] = 17 ms, repetition time [TR] = 1 s, CHESS water suppression, 1,024 averages) were obtained with Bruker's ParaVision acquisition software. The integral of the IMCL signal (1.3 ppm) was related to that of total creatine (tCr; 3.05 ppm; program MRUI 97.2, cf. www.mrui.uab.es). The IMCL/tCr ratio corresponded to the total muscle IMCL value. In all cases, a clear distinction between EMCL and IMCL was possible. As the IMCL/tCr ratio did not change on using a relaxation delay TR of 2 s instead of 1 s ( $n = 4$ ), the influence of relaxation time changes on observed IMCL values could be excluded. The tCr values for the M. soleus and M. tibialis anterior were determined in obese ZDF rats at 8 weeks and 15 months of age ( $n = 8$  each). In both muscles, tCr concentrations proved to be constant (soleus  $89 \pm 1.1$  and  $81 \pm 1.7$   $\mu$ mol/g dry wt; tibialis  $136 \pm 2.2$  and  $132 \pm 1.0$   $\mu$ mol/g dry wt, respectively) and therefore to be a good reference for quantification of IMCL.

For assessing hepatic fat content, the rats were placed prone with their upper abdomen on top of the detecting surface coil. Volumen-localized, respiration-triggered  $^1$ H-MR spectra were obtained without water suppression (TE = 28 ms, TR = 1 s, 8 mm $^3$ ). Fat content was expressed as the ratio of the fat to water signal in percentage.

### Euglycemic-hyperinsulinemic glucose clamp study (protocols B and C).

The study was performed as described previously (34). Briefly, overnight-fasted rats were anesthetized with pentobarbital (priming bolus 60 mg/kg i.p. plus infusion subcutaneously at  $\sim$ 20 mg  $\cdot$  kg $^{-1}$   $\cdot$  h $^{-1}$ ), and their temperature was kept at 37.5°C. Glucose and insulin were infused through left jugular and femoral vein catheters.

Studies lasted 240 min. During a 120-min baseline period, blood glucose was determined every 15 min. Then, insulin was administered as a bolus (48 mU  $\cdot$  kg $^{-1}$   $\cdot$  min $^{-1}$ ) for 5 min, followed by a constant intravenous infusion at 4.8 mU  $\cdot$  kg $^{-1}$   $\cdot$  min $^{-1}$  for an additional 115 min. Blood drawn from the tip of the tail was then measured for glucose every 5 min, and 15% D-glucose was infused to maintain euglycemia at  $\sim$ 5 mmol/l. At 120 and at 240 min, additional blood samples were drawn from the right jugular vein and placed in K-EDTA



**FIG. 1. A:** Representative volume of selective in vivo  $^1$ H-MR spectra at 7 Tesla of the M. tibialis anterior of an obese ZDF rat (20 weeks old,  $\sim$ 470 g) derived from different volume sizes: 4 mm $^3$ , 1,024 averages (a); 8 mm $^3$ , 512 averages (b); and 18 mm $^3$ , 256 averages (c). Reduction of the selected volume diminishes the EMCL signal at 1.5 ppm and facilitates the quantification of IMCL at 1.3 ppm. Spectra were referenced to the tCr resonance at 3.05 ppm. **B:** Representative volume of selective in vivo  $^1$ H-MR spectra at 7 Tesla of the M. tibialis anterior of a lean ZDF rat (a) and an obese ZDF rat (b). Both rats were  $\sim$ 8 weeks old. Volume: 8 mm $^3$ . Note the elevated IMCL-to-tCr integral ratio in the spectrum of the obese ZDF rat as compared with the lean littermate.

tubes, which were immediately centrifuged at 6°C at 5,000 rpm followed by determination of plasma FFA within 60 min; plasma aliquots were frozen for insulin determinations. Animals were killed by pentobarbital overdose. Whole-body IS was calculated as the mean glucose infusion rate (GIR) during the last 60 min of the clamp study.

**Monitoring of metabolic plasma parameters (protocol C).** Monitoring of plasma FFAs, triglycerides, cholesterol, and insulin was performed in non-fasted animals. Blood samples were obtained retro-orbitally during short-term isoflurane anesthesia as indicated in the figures. All blood samples were placed in K-EDTA tubes. Blood was immediately centrifuged at 6°C at 5,000 rpm for determination of plasma parameters.

**Analytical procedures.** Standard procedures were used to determine blood glucose, FFAs, triglycerides, cholesterol, and tCr (35). Plasma insulin concentrations were assayed with an antibody radioimmunoassay kit obtained from Linco (St. Charles, MO).

**Statistical analysis.** Data are presented as mean  $\pm$  SE. Statistical differences were determined using a paired or unpaired bilateral *t* test or a one-way ANOVA followed by a post hoc analysis with Bonferroni's correction when testing for differences between three experimental groups using the software package SigmaStat (Jandel, Erkrath, Germany).  $P < 0.05$  was considered to be statistically significant.

## RESULTS

**MRS.** Up to now, all reported MRS studies on IMCL have been performed on human volunteers or patients (17–21,25–32); however, for preclinical studies, rat models are widely used in biomedical research. The obtained rat spectra are very similar to those known from human studies exhibiting the two distinct signals of EMCL and IMCL (Fig. 1A). In rats, the contribution of EMCL can often be strongly reduced or completely eliminated by accurate positioning of the volume of interest within the muscle mass and by reducing its size. Working with voxels of  $\sim$ 8

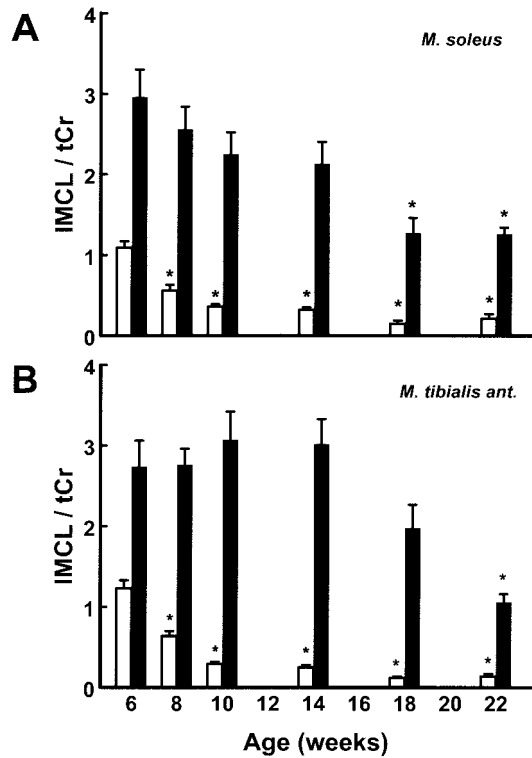


FIG. 2. In vivo <sup>1</sup>H-MRS data for IMCL/tCr in *M. soleus* (A) and *M. tibialis anterior* (B) in lean (white bars) and obese (black bars) ZDF rats (protocol A). Values are means ± SE, n = 7–8. For the respective age, all values were significantly different (P < 0.001) between lean and obese rats. \*P < 0.001 vs. value at 6 weeks of age for the respective rat group.

mm<sup>3</sup> size, IMCL was better quantified. Also, these voxels allow one specific muscle to be sampled selectively. Thus, we monitored the variations of IMCL levels over a period of 4 months selectively in a slow-twitch muscle with high oxidative capacity, the *M. soleus*, and in a fast-twitch muscle with high glycolytic capacity, the white *M. tibialis anterior*.

**Protocol A.** Already in the prediabetic state at 6 weeks of age, obese ZDF rats exhibited significantly higher IMCL levels in *M. soleus* and in *M. tibialis anterior* than their lean littermates (Figs. 1B and 2). Subsequently, in all animals, IMCL content decreased in both muscles: in lean ZDF rats at a relatively constant rate throughout the study period, an effect related to aging; in obese animals, a pronounced decline in IMCL at a higher age (18 and 22 weeks) was noted, especially in the *M. tibialis anterior*, which might reflect the change in metabolism as a result of the overt diabetic state at that age.

TABLE 1

Mean values of GIR (mg · kg<sup>-1</sup> · min<sup>-1</sup>) and FFA (mmol/l) during euglycemic-hyperinsulinemic glucose clamp studies in lean and obese ZDF rats

Age (weeks)	ZDF rat groups	GIR	FFA (mmol/l)	
			Basal	Clamp
6–7	Lean	20.2 ± 0.8	0.58 ± 0.06	0.06 ± 0.01
6–7	Obese	2.7 ± 0.8*	1.07 ± 0.02*	0.52 ± 0.04*
22–24	Lean	14.4 ± 0.5	0.45 ± 0.03	0.04 ± 0.01
22–24	Obese	1.6 ± 0.6*	0.64 ± 0.05†	0.15 ± 0.03†
22–24	Obese+RGZ	6.7 ± 0.8 <sup>‡</sup>	1.05 ± 0.06‡	0.26 ± 0.04§

Data are means ± SE; n = 5–8; \*P < 0.001, †P < 0.05 vs. age-matched lean control; ‡P < 0.001, §P < 0.05 vs. age-matched obese control.

**Protocol B.** At the age of 6–7 weeks, obese ZDF rats displayed marked IR compared with their lean littermates, demonstrated by lower GIR and by elevated levels of FFA, which were not as suppressible during insulin infusion as in lean rats (Table 1). Insulin-induced antilipolysis during the clamp study was 90% in lean and only 51% in obese rats. The impaired IS in obese rats is in line with the increased IMCL content at that age (Fig. 2).

**Protocol C.** The metabolic plasma parameters of lean and obese ZDF rats were monitored over a period of 4 months (Fig. 3). At the age of 6 weeks, there was no difference between lean and obese animals regarding blood glucose levels and plasma FFA, whereas plasma triglyceride, insulin, and cholesterol levels were significantly elevated in the latter. Hyperinsulinemia and hyperlipidemia reflect the early insulin-resistant, prediabetic condition in this animal model, in line with the data derived from <sup>1</sup>H-MRS (protocol A) and glucose clamp studies (protocol B). As the animals aged, the metabolic plasma parameters remained constant in lean controls, whereas they worsened in the obese ZDF rats, reflecting their progression to an overt diabetic state. The deterioration of their metabolic state throughout the study was evidenced by rapidly increasing plasma triglyceride levels, gradually decreasing plasma insulin concentrations, and increasing blood glucose levels as well as a gradual body weight loss. The transition to overt diabetes culminated at ~14–16 weeks of age.

All initial metabolic values in the RGZ-treated group were close to those of the obese control group, as expected. RGZ administration resulted in significantly decreased plasma insulin and triglyceride levels as compared with the obese controls during the treatment period. Hyperglycemia was prevented; blood glucose levels were indeed equivalent to those of lean control rats as reported earlier (36). However, starting at the age of 16 weeks, FFA and insulin levels increased, probably as a result of the increased body fat mass as reflected by the increased body weight gain (Fig. 3).

Hepatic fat content at 17 weeks determined in <sup>1</sup>H-MR spectra already reflected the remodeling effect of RGZ on adipose tissue (cf. Fig. 4) (34). Obese ZDF rats showed an approximately sevenfold higher hepatic fat content than their lean littermates (10.4 ± 0.3 vs. 1.4 ± 0.5%; P < 0.001). RGZ treatment reduced hepatic fat content to 6.1 ± 0.6% (P < 0.001 vs. obese group). The redistribution of fat depots throughout the body on RGZ treatment led also to an increased amount of interspersed adipocytes within muscle tissue, resulting in a strong overlap of the EMCL and the IMCL signals.

At 16 weeks of age, IMCL levels were significantly

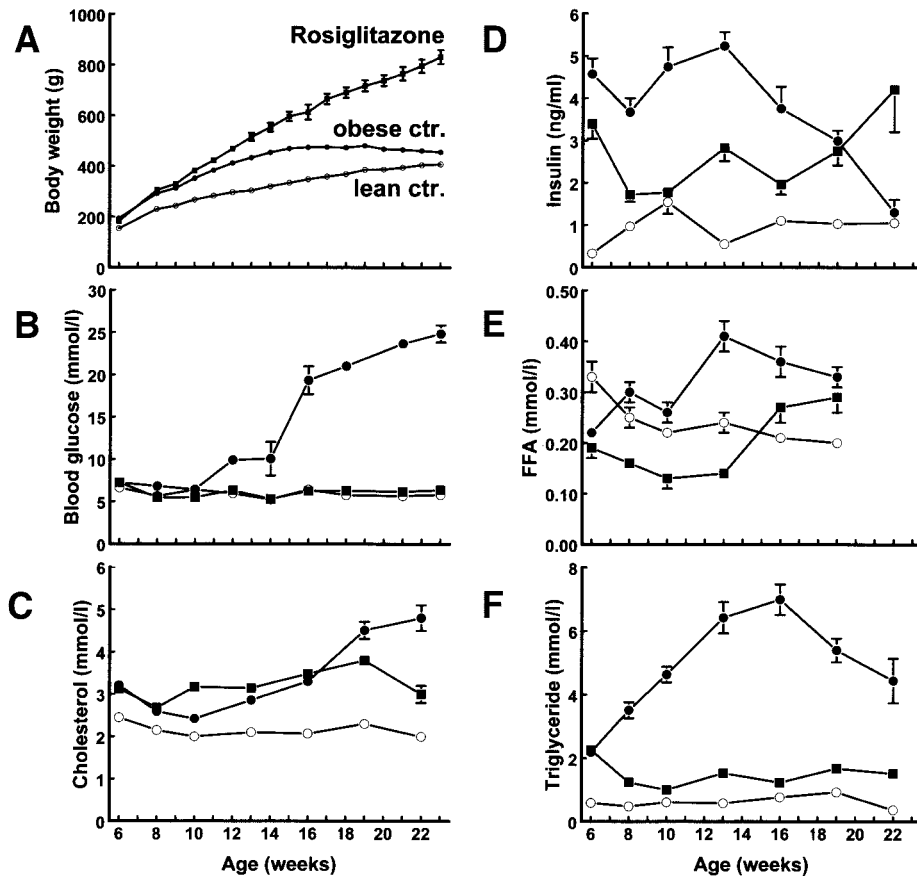


FIG. 3. Body weight (A), blood glucose (B), cholesterol (C), insulin (D), FFA (E), and triglyceride (F) in lean control (white circles), obese control (black circles), and obese RGZ-treated (black squares) ZDF rats (protocol C). Plasma parameters were obtained during nonstarved conditions. Values are means  $\pm$  SE,  $n = 7-8$ .

decreased in RGZ-treated rats compared with obese controls (Fig. 5). IMCL did not decrease any further in the course of the treatment. However, at the age of 22 weeks, IMCL levels in obese control rats had decreased and were no longer significantly different from those in RGZ-treated rats. This observation was in line with the results from protocol A (Fig. 2), suggesting an impact of the manifest diabetes with its variety of metabolic disturbances on IMCL content in obese ZDF rats.

IS was determined when animals were 22–24 weeks old.

Obese untreated ZDF rats were markedly insulin resistant compared with their lean littermates, as demonstrated by the lower GIR and by elevated levels of FFA (Table 1). Both lean and obese ZDF rats were less insulin sensitive than at 6 weeks of age (Table 1). This decline in IS relates probably to aging, in obese controls additionally to diabetes-related effects, such as a hyperglycemia-induced IR (37,38). In RGZ-treated obese ZDF rats, IS improved markedly as evidenced by increased GIR. Basal FFA levels were elevated as a result of their extreme obesity but were suppressible to a similar extent as in the obese controls (75 vs. 76%).

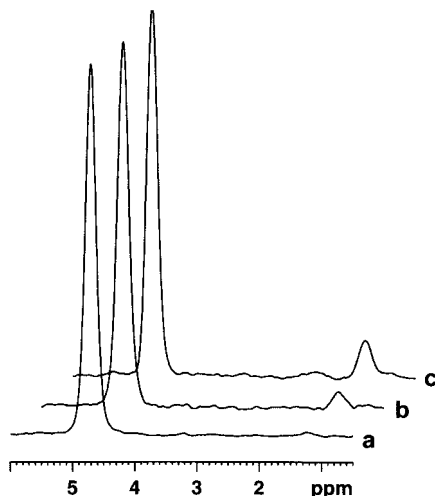


FIG. 4. Representative in vivo  $^1\text{H}$ -MR spectra of liver in lean control (a), RGZ-treated obese (b), and obese control ZDF rat (c) at the age of 17 weeks (protocol C). Spectra show water signal at 4.7 ppm and fat resonance at 1.3 ppm.

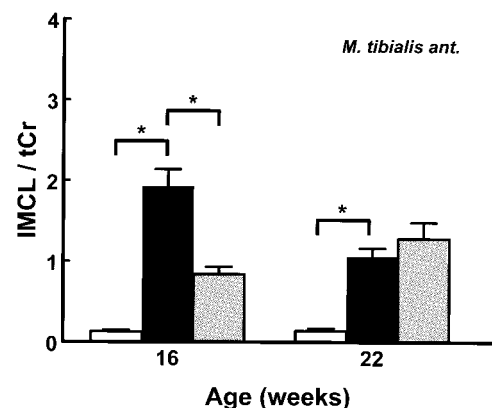


FIG. 5. In vivo  $^1\text{H}$ -MRS data for IMCL/tCr in *M. tibialis ant.* in lean control (white bars), obese control (black bars), and obese RGZ-treated (grey bars) ZDF rats at the ages of 16 and 22 weeks (protocol C). Values are means  $\pm$  SE,  $n = 6-8$ . \* $P < 0.001$ .

## DISCUSSION

The selective, noninvasive detection of the two lipid pools in muscle tissue, IMCL and EMCL, by localized  $^1\text{H-MRS}$  methods is based on the anisotropic arrangement of muscle fibers in the body (25–27). Adipocytes within muscle tissue appear arranged along the muscle fasciae and experience changes in magnetic susceptibility as these fasciae change their orientation to the main magnetic field  $B_0$ . Therefore, the signals of adipocytic lipids will be affected in their resonance frequency in an orientation-dependent manner. The IMCL deposits, however, exhibit in histological slices a spherical structure, which will show no spatial dependence on the magnetic field direction (26). This orientational dependence is essential for the separate observation of the two lipid pools by  $^1\text{H-MRS}$ . Whereas in human  $^1\text{H-MR}$  spectra the signals of EMCL and IMCL always appear together and overlap, in rats the selective detection of IMCL is often possible.

Recently,  $^1\text{H-MRS}$  of IMCL has received increasing attention, because IMCL levels in humans have been shown to correlate with IR (20,21,28–32) but to depend also on other effects, such as diet and exercise (16–18). The inverse relationship between IMCL levels in the isolated *M. soleus* ex vivo and IS in normal Sprague-Dawley rats was investigated by McGarry's group (13). IR was induced by high-fat feeding, and the concomitant administration of etomoxir, a CPT-1 inhibitor, blocked lipid oxidation in muscle and thereby contributed to IMCL accumulation.

The inbred ZDF rat used in our study is an established adult-onset diabetic model with a Zucker background and the "fa" gene (39). Obese ZDF rats are insulin resistant, and male rats spontaneously progress to overt diabetes at the age of 10–12 weeks. The present study shows that IMCL can be repeatedly monitored in ZDF rats by in vivo  $^1\text{H-MRS}$ . There was an age-related decline in IMCL during the study period, similar to that observed in Wistar rats (40). This decline was relatively linear in lean rats. In obese ZDF rats, however, IMCL values in the *M. tibialis anterior* remained relatively constant on an elevated level. At the age of 18–22 weeks followed a marked decline when animals turned to an overt diabetic state. One possible explanation for the IMCL decline in old diabetic obese ZDF rats is the diabetic condition itself associated with a catabolic lipid metabolism not present in young prediabetic obese ZDF rats. The onset of this catabolic metabolism with the onset of diabetes was also reflected by a decreased rather than sustained body weight development. During this late state of diabetes, plasma triglycerides and insulin also declined. In human studies, it was demonstrated that fatty acids in the presence of hyperinsulinemia were essential for the increase in IMCLs (18). Thus, the decline of both parameters during our study in obese diabetic ZDF rats at higher ages could possibly explain the decrease in IMCL. In the same way as in lean rats the increasing age may additionally affect the IMCL decline in obese ZDF rats.

Thus, this ZDF rat study confirmed the relationship between IS and IMCL content demonstrated in humans (20–22,28–32). During the whole study period of 4 months, the detected IMCL concentrations were significantly increased in obese ZDF rats as compared with their lean littermates. In line with elevated IMCL concentrations in

obese rats, the GIR as a marker for whole-body glucose utilization was significantly lower in young prediabetic obese and in old diabetic obese ZDF rats as compared with their lean littermates of the same age. However, there is no simple inverse correlation between IMCL and IS as demonstrated by the fact that young rats exhibited a higher IS despite having higher IMCL levels than older rats. Therefore, age matching is an essential requirement when using IMCL as a biomarker for IR.

RGZ, a thiazolidinedione (TZD), is used for the treatment of insulin-resistant states in type 2 diabetes (41). TZD are ligands for the PPAR- $\gamma$ , which is expressed primarily in adipose tissue (42). TZD modulates adipocyte differentiation, enhances IS, decreases circulating lipids (43–45), and promotes a redistribution of fat from nonadipose tissue back into the adipocytes, thus alleviating IR in skeletal muscle. Recently, the improved glucose homeostasis as a result of TZD treatment has been associated with reduced tissue lipid availability (45). RGZ treatment of obese ZDF rats normalized plasma triglycerides and reduced transiently plasma FFAs as well as insulin levels and prevented the onset of diabetes while increasing body weight gain (36). Although this gain is predominantly due to increased body fat mass (46), metabolic plasma parameters including plasma lipids were transiently almost normalized; hepatic fat content was significantly reduced as well. However, starting at the age of 16 weeks, FFAs and insulin increased, which might reflect the start of a decompensation of metabolic control as a result of the extraordinary increase in body fat mass and body weight. Prolonged studies are necessary for clarification.

In line with its insulin-sensitizing properties, RGZ caused a marked reduction of IMCL content in *M. tibialis anterior* at the age of 16 weeks. This lowered IMCL level was also maintained at the age of 22 weeks. However, at that latter age, IMCL values in obese control and obese RGZ-treated ZDF rats were not significantly different, although RGZ caused a significant improvement of IS. In this context, it is important to stress that at that age of 22–24 weeks, we were comparing obese diabetic ZDF rats exhibiting a diabetes-induced catabolic metabolism and hyperglycemia-induced IR, e.g., as a result of increased carbon flux through the hexosamine pathway (37,38), with obese nondiabetic ZDF rats as a result of the RGZ treatment and displaying an anabolic metabolism as reflected by body weight gain. As we did not measure hepatic glucose production by tracer techniques during the clamp, we cannot assign the improved IS in RGZ-treated rats exclusively to an increase in muscle glucose disposal.

Recently published results in RGZ-treated patients with type 2 diabetes surprisingly demonstrated no change in IMCL content (47). These results are presently not fully understood. They could be related to the far lower RGZ dose applied in humans as compared with our rat study. Another explanation for these unexpected human results might be the study design; we started the RGZ treatment as an early intervention of IR in ZDF rats, whereas the human study involved the therapy of patients with overt type 2 diabetes in a later disease state.

From our study, we conclude that IMCL could serve as a biomarker for IR in ZDF rats and that monitoring of IMCL signals by  $^1\text{H-MRS}$  in rats is a useful method for the

longitudinal characterization of new drugs that affect muscle lipid metabolism in relation to IS. The effect of diabetes itself on IMCL levels in insulin-resistant animals requires additional investigation as well as the contribution of the endogenous glucose production for whole-body IS after RGZ treatment.

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