Reduction of Intramyocellular Lipid Following Short-Term Rosiglitazone Treatment in Zucker Fatty Rats: An In Vivo Nuclear Magnetic Resonance Study

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The aim of the study was to characterize the effects of rosiglitazone, an oral insulin sensitizer, on intramyocellular lipid (IMCL) content in tibialis anterior muscle and whole body lipid deposition in Zucker fatty rats using in vivo ^{1}H nuclear magnetic resonance (NMR) spectroscopy. The IMCL/EMCL (extramyocellular) ratio was significantly lower in the rosiglitazone (FRSG) group at 7, 14, 21, and 28 days of treatment at 3 mg/kg/d (0.04 ± 0.01, 0.09 ± 0.03, 0.11 ± 0.02, and 0.07 ± 0.02, respectively) versus baseline (0.43 ± 0.12, $P < .01$ *v* all time points), whereas there was no difference in the control (FC) group at these time points (0.31 ± 0.08, 0.36 ± 0.08, 0.40 ± 0.14, and 0.49 ± 0.18, respectively) versus baseline (0.37 ± 0.07). Absolute IMCL content was also lower at 28 days in the FRSG (0.41 ± 0.09 μmol/g) versus FC (2.13 ± 0.40 μmol/g, $P < .005$) group. To further characterize the temporal nature of this change, the IMCL/EMCL ratio was examined in the FRSG group on each of the first 4 days of treatment, and a steady decline was observed (0.38 ± 0.12, 0.21 ± 0.08, 0.12 ± 0.04, 0.09 ± 0.04, 0.05 ± 0.03 at baseline and days 1, 2, 3, and 4 respectively, $P < .05$ baseline *v* all time points). To examine the relationship between IMCL and insulin sensitivity, a euglycemic-hyperinsulinemic clamp and IMCL measurement was performed on 7-day treated FRSG and FC groups. There was a negative correlation between absolute IMCL content and glucose infusion rate ($r = -0.47$, $P < .04$). The FRSG and the FC groups had similar whole body lipid content (expressed as a percentage of whole body water content) at baseline (48% ± 5% and 44% ± 2%, respectively), but the value was greater in the FRSG group following 28 days of treatment (103% ± 4 *v* 84% ± 6%, respectively, $P < .02$). In summary, there was a rapid (days) and pronounced reduction (↓ ~70%) in IMCL content in tibialis anterior muscle following rosiglitazone treatment. Additionally, the increase in whole body lipid in the FRSG group suggests that there was increased adipocyte lipid storage following long-term rosiglitazone treatment. These results support the hypothesis that rosiglitazone indirectly increases peripheral insulin sensitivity by decreasing adipocyte lipolysis, thereby lowering IMCL content.

TYPE 2 DIABETES is characterized by, among other things, skeletal muscle insulin resistance.[1,2] Additionally, risk factors such as obesity and dyslipidemia are correlated with whole body insulin resistance.[3,4] Elevated plasma triglyceride (TG) and fatty acids (FFA) have been linked with defects in skeletal muscle glucose uptake via a number of different mechanisms including inhibition of insulin receptor substrate-1 (IRS-1)–associated phosphatidylinositol 3 (PI-3) kinase activation,[5,6] protein kinase C (PKC) activation,[7,8] glycogen synthase downregulation,[9] and fat oxidation–mediated inhibition (Randle cycle).[10] Both acute elevation of plasma FFA via exogenous administration in man[5,11] and chronic elevation of plasma FFA and TG via high-fat feeding in rodents[12-14] have been shown to induce skeletal muscle insulin resistance.

Kraegan et al showed that the chronic elevation of dietary fat was not only associated with skeletal muscle insulin resistance, but also with an increase in muscle TG content as measured in biopsies.[14] This study was followed by numerous others which similarly showed a negative correlation between skeletal muscle TG, long chain acyl-coenzyme A (acyl-CoA), or diacylglycerol content and insulin-stimulated glucose uptake.[13,15,16] Traditionally, biochemical or gas chromatographic methods are used to analyze lipid metabolites in tissue biopsy extracts. The inherent limitation to measuring the metabolically active pool in gross skeletal muscle tissue is the inability to discriminate between intramyocellular (within muscle fibers) and extramyocellular (between muscle fibers—typically in adipocytes) pools of TG. In vivo ^{1}H nuclear magnetic resonance (NMR) spectroscopy, by virtue of different magnetic field environments associated with the different lipid pools when oriented along the magnetic field, allows for discrimination of intramyocelluar lipid (IMCL) from extramyocellular lipid (EMCL) at magnetic fields as low as 1.5T.[17] To date, ^{1}H NMR spectroscopy has been used to establish a negative correlation between IMCL and insulin sensitivity in lean individuals[18,19] and offspring of type 2 diabetics.[20,21] Additionally, IMCL accumulation following increased dietary lipid consumption[22] or after acute elevation of plasma FFA[22-24] has been observed using ^{1}H NMR spectroscopy.

Rosiglitazone, a member of the thiazolidinedione (TZD) class of oral antihyperglycemic agents for the treatment of type 2 diabetes, is a potent peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist. Studies in animal models of type 2 diabetes show that rosiglitazone improves glycemic control by enhancing insulin-stimulated whole body glucose disposal.[25,26] The increase in whole body insulin sensitivity is in part due to an increase in insulin action in skeletal muscle.[25] Although skeletal muscle expresses low levels of PPAR-γ[27] and direct actions of TZDs on muscle glucose metabolism in vitro have been reported,[28,29] the precise mechanism of action of TZDs in skeletal muscle is unclear. Much evidence exists to suggest that the improvement in muscle insulin sensitivity may be an indi-

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rect consequence of activation of PPAR-γ in fat, a tissue in which the receptor is abundantly expressed. Activation of PPAR-γ in fat results in decreased adipocyte lipolysis and hence FFA availability.[16,25,26,30] This in turn, would lead to a subsequent decrease in IMCL and diacylglycerol content.[16]

Therefore the aim of the study was to temporally characterize the IMCL content in Zucker fatty rats (model of insulin resistance) before and following rosiglitazone treatment (0 to 28 days). Additionally, euglycemic-hyperinsulinemic clamps were performed in order to correlate the IMCL content with whole body insulin sensitivity. Whole body fat measurements were made in order to further address the PPAR-γ–mediated mechanism by which rosiglitazone increases whole body glucose disposal.

MATERIALS AND METHODS

Animals

Zucker obese (fa/fa) rats (Harlan, Indianapolis, IN) were housed in an environmentally controlled room with a 12-hour light/dark cycle. Obese rats (8 to 10 weeks old) were placed on a pair-fed chow diet (#5L35, LabDiet, Richmond, IN) consisting of 100 kcal/d in order to minimize inter-rat dietary variations. Rats were placed into 2 groups: fatty-rosiglitazone (FRSG) and fatty-control (FC). The FRSG group received 3 mg/kg rosiglitazone (rosiglitazone maleate) via oral gavage once daily and the FC group received vehicle (sterile water) for a period of 4 days (n = 5, FRSG group), 7 days (n = 8, FRSG and FC groups), or 28 days (n = 5, FRSG group; n = 7, FC group). In experiments where a euglycemic-hyperinsulinemic experiment was performed, rats were chronically catheterized (carotid artery and jugular vein) 3 days prior to the experiment.

In Vivo Experiments

Rats were anesthetized with 2.0% isoflurane (Abbott Laboratories, Chicago, IL) during the NMR experiments. In experiments where a euglycemic-hyperinsulinemic clamp was coupled with NMR experiments, the NMR was performed first followed by the glucose-insulin clamp in the awake, unrestrained rat. After an overnight fast, a glucose-insulin clamp was performed using a primed-continuous insulin infusion (10 mU/kg/min, Humulin Regular, Eli Lilly, Indianapolis, IN), which was begun 2.5 minutes prior to a variable glucose (20% wt/vol dextrose) infusion. Plasma glucose concentrations were maintained between 6 and 8 mmol/L for the duration of the experiment (120 minutes). Blood samples were drawn at 0, 7.5, and 15 minutes, and every 15 minutes thereafter for immediate assessment of plasma glucose concentration. At the end of the NMR time course measurements, rats were anesthetized with Nembutal (Abbott Laboratories) at 50 mg/kg intravenously. Superficial skin was rapidly removed from the left hindquarter followed by in situ freeze clamping of the tibialis anterior and gastrocnemius muscle. Rats were euthanized with a lethal dose of Nembutal.

In Vivo NMR Spectroscopy

All in vivo ^{1}H NMR spectroscopic and imaging experiments were performed on a Bruker (Billerica, MA) ABX system (horizontal/40 cm diameter bore, 4.7T field strength magnet). After an overnight fast, IMCL and EMCL measurements were performed on the tibialis anterior muscle using a ^{1}H radiofrequency surface coil (42 mm diameter) tuned to 200.21 MHz. The rat was positioned prone to the radiofrequency coil/platform assembly (horizontal in plane) and the right hind limb was placed so that the lower hind limb was placed in magnet isocenter. Transverse and coronal gradient echo scout images (TR = 500 ms, TE = 7 ms) were acquired to achieve proper placement of the spectroscopic region of interest in the tibialis anterior muscle. Intra-rat voxel (cube volume) placement was preserved using the following anatomical landmarks: 7.5 mm distal to the head of the tibia in the coronal slice, and ~0.5 mm lateral to the tibia in the transverse slice. The tibialis anterior muscle was used for spectroscopic purposes, because the muscle fiber orientation along the magnetic field lends itself to optimal discrimination of IMCL (1.3 ppm) from EMCL (1.5 ppm) NMR peaks. The fiber orientation along the magnetic field is critical in obtaining spectral resolution between the IMCL and EMCL NMR peaks.[31] ^{1}H point resolved spectroscopy (PRESS) spectroscopy (TE = 22 ms, TR = 2,000 ms, NS = 512) with chemical shift selected (CHESS) water suppression was performed over a 3 × 3 × 3 mm voxel in the tibialis anterior muscle. Whole body lipid measurements were performed in fed rats using a 10-cm diameter whole body ^{1}H resonator and a single 90-degree hard pulse. NMR frequencies for the IMCL and EMCL measurements were referenced to creatine/phosphocreatine (Cr/PCr) at 3.07 ppm and in the whole body lipid measurement, water was referenced to 4.7 ppm. For IMCL, EMCL, and Cr/PCr measurements, ^{1}H NMR spectra were processed using a Gaussian filter, and baseline flattening before automated peak deconvolution by a simplex routine (Nuts NMR processing software, Acorn NMR Inc, Fremont, CA). For whole body water and lipid measurements, peaks were integrated.

Tissue Triglyceride Measurement

Skeletal muscle TG content was determined using a method adapted from Storlein et al[13] and Frayn and Maycock.[32] Approximately 0.3 to 0.5 g of frozen tissue was ground under liquid N_2 using a mortar and pestle. All measurements were made in triplicate by placing a weighed portion of the powdered muscle into 3 separate tubes. Chloroform/methanol (3 mL, 2:1 vol/vol) was added to each tube before homogenizing (~10,000 rpm) using a T25 Ultra-Turrax homogenizer (IKA, Wilmington, NC) over ice for approximately 1 minute. Tubes were shaken for 4 hours at room temperature before adding 2 mL of 1 mol/L H_2SO_4. Samples were shaken and subsequently centrifuged (1,000 rpm for 10 minutes) to separate phases. The organic bottom layer containing triglyceride was separated and was assumed to be 2 mL volume. This portion was used in the plasma triglyceride assay. Enzymatic assay (kit #336-10, Sigma Diagnostics, St Louis, MO) was run using a Spectra Max Plus spectrophotometer (Molecular Devices, Sunnyvale, CA) to measure triglyceride content. Absolute IMCL concentration was extrapolated from the NMR-derived IMCL/EMCL ratio and whole muscle TG concentration.

Tissue Histology

Skeletal muscle tissue (gastrocnemius, soleus, and tibialis anterior) was harvested after a 7-day treatment period in FRSG and FC rats for histological staining of IMCL. Portions of muscle tissue were trimmed to 1 cm in length after removal of connective tissue and superficial fatty deposits. The tissue was fixed in 10% formalin solution and then transferred into 30% sucrose in phosphate-buffered saline (PBS). After embedding in optimum cutting temperature (O.C.T) compound (Sakura, Torrance, CA), samples were cut parallel to the fiber orientation at 5 μm on a cryostat. Frozen samples were stained with Oil red O for lipid content. Sample analysis was performed using an Olympus BX60 microscope (Olympus Optical Company, Melville, NY) with a 20 to 40x magnification. The images were recorded using a mounted 35-mm camera.

Analytical Procedures

Plasma glucose concentrations were measured using a 2300 STAT PLUS analyzer (Yellow Springs Instrument, Yellow Springs, OH). Plasma insulin was measured using an enzyme-linked immunosorbent assay (ELISA) immunoassay (ALPO Diagnostics, Windham, NH).

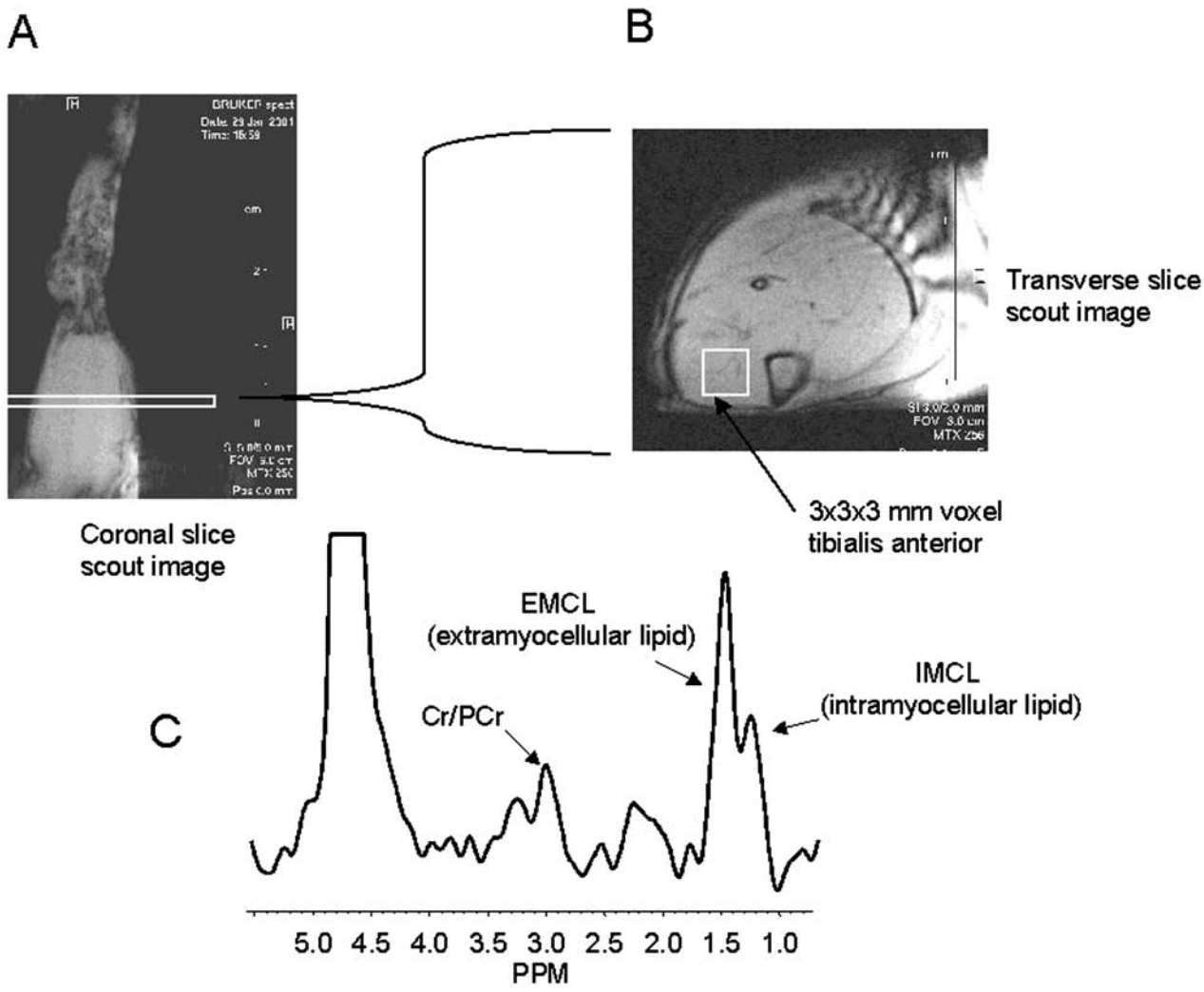

Fig 1. Volume-selective ^{1}H NMR spectroscopy in tibialis anterior muscle. (A) Coronal and (B) transverse gradient echo scout images (TR = 500 ms, TE = 7 ms) were acquired to achieve proper placement of the spectroscopic region of interest in the tibialis anterior muscle. (C) ^{1}H PRESS NMR spectroscopy (TE = 22 ms, TR = 2,000 ms) with CHESS water suppression was performed over a 3 × 3 × 3 mm voxel in the tibialis anterior muscle. Cr/PCr, EMCL, IMCL peaks appear at 3.1, 1.5, and 1.3 ppm, respectively.

Plasma triglyceride concentrations were determined enzymatically using an Olympus AU600 analyzer.

Statistical Analysis

All data are reported as the mean ± SEM. Student's 2-tailed *t* test was performed on data to determine significance at a minimum $P < .05$ threshold between the FRSG and FC groups. When analyzing repetitive measure data, analysis of variance (ANOVA) was performed to determine significance at a minimum $P < .05$ threshold. A multiple comparison Newman-Keuls post-hoc test was used when necessary to determine significance at different time points.

RESULTS

Weight Gain, Plasma Triglyceride, and Insulin

As a result of the pair feeding protocol, there were no differences in final weight or weight gain (Δ) between the FC and FRSG groups after 7-day (355 ± 9, Δ = 32 ± 3 and 366 ± 8, Δ = 33 ± 3 g) or 28-day (401 ± 5, Δ = 120 ± 6 and 388 ± 4, Δ = 124 ± 2 g) rosiglitazone treatment, respectively. Plasma TG concentration was lower in the FRSG versus FC group following 7-day (125 ± 16 *v* 231 ± 31 mg/dL, respectively, $P < .005$), and 28-day (291 ± 37 *v* 498 ± 71 mg/dL, respectively, $P < .05$) treatment. Although not significant, there was a trend toward lower basal insulin in the FRSG versus FC group at the 7-day (2.2 ± 0.6 *v* 3.1 ± 0.5 ng/mL and 28-day (2.1 ± 0.7 *v* 3.5 ± 0.8 ng/mL) treatment time points.

IMCL Measurement

Coronal and transverse slice images of the rat hind limb are shown in Fig 1A and B, respectively. Scout images such as those shown in Fig 1 were used to position the spectroscopic voxel (3 × 3 × 3 mm) of interest in the tibialis anterior muscle. The ^{1}H spectrum obtained from the voxel is shown in Fig 1C.

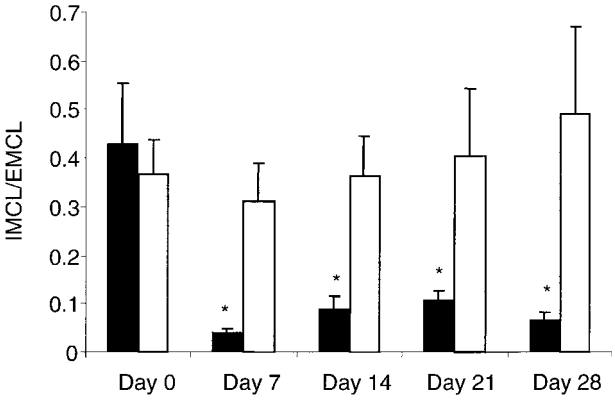

Fig 2. Tibialis anterior IMCL/EMCL ratio during 28-day rosiglitazone treatment. IMCL/EMCL ratio in FRSG (■, n = 5) and FC (□, n = 7) groups at baseline (day 0), and days 7, 14, 21, and 28 is presented. Data are reported as mean ± SEM. *$P < .01$ *v* baseline.

The IMCL, EMCL, and Cr/PCr NMR peaks appear at 1.3, 1.5, and 3.1 ppm, respectively. A significant EMCL peak was observed in all Zucker fatty rats at all time points.

The IMCL/EMCL peak ratio was significantly lower in the FRSG group at 7, 14, 21, and 28 days of treatment (0.04 ± 0.01, 0.09 ± 0.03, 0.11 ± 0.02, and 0.07 ± 0.02, respectively, Fig 2) versus baseline (0.43 ± 0.12, $P < .01$ *v* all time points), whereas there was no difference in the FC group at these time points (0.31 ± 0.08, 0.36 ± 0.08, 0.40 ± 0.14, and 0.49 ± 0.18, respectively, Fig 2) versus baseline (0.37 ± 0.07). Although absolute TG content in tibialis anterior was the same in the FRSG and FC groups (6.7 ± 0.9 μmol/g and 7.7 ± 0.9 μmol/g) following 28 days of treatment, absolute IMCL content was lower in the FRSG group (0.41 ± 0.09 μmol/g) versus the FC group (2.13 ± 0.40 μmol/g, $P < .005$). There was no difference in EMCL in the FRSG group (6.3 ± 0.9 μmol/g) versus the FC group (5.5 ± 0.8 μmol/g). The absence of an absolute decrease in TG content in the presence of an 80% reduction in IMCL content in tibialis anterior muscle following 28-day rosiglitazone therapy was due to the very high EMCL/IMCL ratio observed (~10- to 20-fold). However, in the gastrocnemius muscle, there was a decrease in the absolute TG concentration as measured by biochemical assay in the FRSG versus FC groups (9.4 ± 1.0 *v* 13.2 ± 0.9 μmol/g, $P < .01$, respectively).

The decrease in IMCL/EMCL ratio in the FRSG group was rapid as inferred from Fig 2 where the IMCL/EMCL ratio is the lowest at the 7-day time point. To further characterize temporally when during the first 7 days the decrease occurs, the IMCL/EMCL ratio was examined in the FRSG group on each of the first 4 days of treatment. A steady temporal decline in this ratio was observed (0.38 ± 0.12, 0.21 ± 0.08, 0.12 ± 0.04, 0.09 ± 0.04, 0.05 ± 0.03 at baseline and days 1, 2, 3, and 4, respectively, $P < .05$ at days 1, 2, 3, and 4 *v* baseline, Fig 3). When the IMCL peak was referenced to the Cr/PCr NMR peak (internal concentration standard), the IMCL/(Cr/PCr) ratio decreased from 1.7 ± 0.3 at baseline to 1.5 ± 0.6, 1.0 ± 0.1, 0.4 ± 0.2, and 0.1 ± 0.1 at day 1, 2, 3, and 4, respectively ($P <$ .05 at day 2, 3, and 4 *v* baseline). Although there was a trend for the EMCL/(Cr/PCr) ratio to increase throughout the 4-day period (5.0 ± 1.3, 6.7 ± 1.2, 17.0 ± 7.8, 10.7 ± 4.3, and 13.0 ± 5.7 at baseline and days 1, 2, 3, and 4, respectively), these changes were variable and not significant. At day 4, the tibialis anterior IMCL concentration was 0.60 ± 0.37 μmol/g. These studies demonstrate that within 24 hours of rosiglitazone treatment, the IMCL content begins to decline.

IMCL Versus Glucose Infusion Rate Correlation

Basal plasma glucose (at day 7 prior to the glucose clamp experiment) in the FRSG group (8.4 ± 0.4 mmol/L) was significantly reduced compared to that of the FC group (11.9 ± 0.9 mmol/L, $P < .001$). During the euglycemic-hyperinsulinemic clamp experiment, plasma glucose concentrations were maintained at ~6 to 8 mmol/L. The glucose infusion rate (GIR) at 120 minutes was significantly higher in the FRSG group (34.9 ± 2.6 mg/kg/min) than in the FC group (19.5 ± 3.4 mg/kg/min, $P < .005$). The increased GIR observed in the FRSG group is an indication that insulin sensitivity was in-

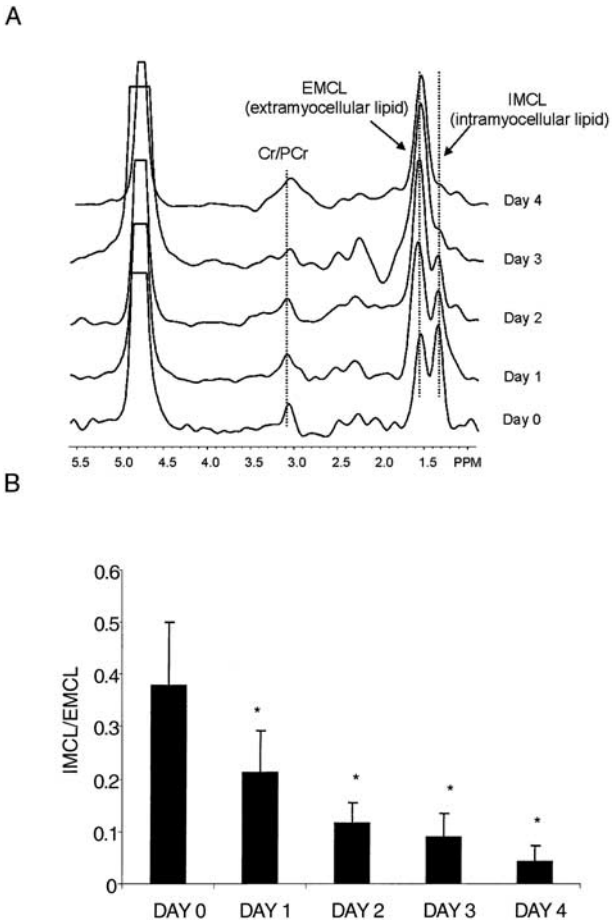

Fig 3. Tibialis anterior IMCL/EMCL ratio during 4-day rosiglitazone treatment. (A) Series of volume-selective ^{1}H NMR spectra taken from a 3 × 3 × 3 mm voxel in the tibialis anterior muscle. The temporal decrease in IMCL (1.3 ppm) over the 4-day period can be observed. (B) IMCL/EMCL ratio in FRSG (■, n = 5) group at baseline (day 0), and days 1, 2, 3, and 4 is presented. Data are reported as mean ± SEM. *$P < .05$ *vs* baseline.

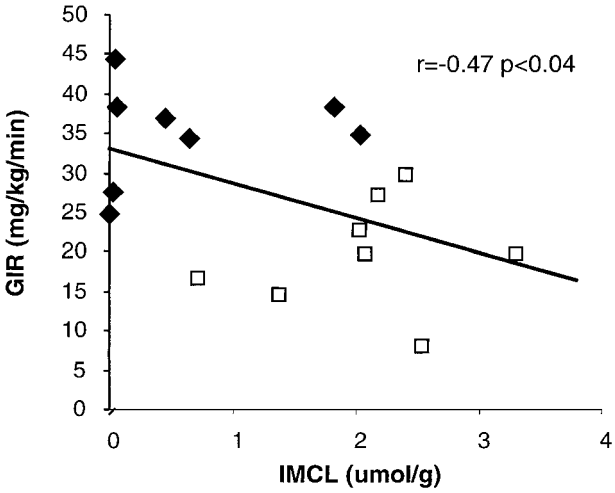

Fig 4. Correlation between insulin sensitivity and IMCL content. Both FRSG (◆, n = 8) and FC (□, n = 8) data are presented. During the euglycemic-hyperinsulinemic clamp, the glucose infusion rate (GIR) at 120 minutes was significantly higher in the FRSG group (34.9 ± 2.6 mg/kg/min) than in the FC group (19.5 ± 3.4 mg/kg/min, $P < .005$). The IMCL content as measured by volume-selective ^{1}H NMR spectroscopy in the tibialis anterior muscle was lower in the FRSG *v* FC group following 7-day rosiglitazone treatment (0.63 ± 0.29 *v* 2.09 ± 0.27 μmol/g, respectively, $P < .005$). The correlation between absolute IMCL content and GIR was $r = -0.47$, $P < .04$.

creased in this group. As expected, the IMCL content in the tibialis anterior muscle was lower in the FRSG versus the FC group following 7-day rosiglitazone treatment (0.63 ± 0.29 *v* 2.09 ± 0.27 μmol/g, respectively, $P < .005$). In combination, these data reflect a significant negative correlation between insulin sensitivity and IMCL content ($r = -0.47$, $P < .04$, Fig 4).

Skeletal Muscle Histology

Qualitative differences in IMCL content in soleus and tibialis anterior muscle in FRSG and FC groups following 7-day rosiglitazone treatment are illustrated in Fig 5. Tissue sections were generated by cutting parallel to the muscle fiber orientation and staining for lipid content with Oil red O. The images represent regions of tissue with significant lipid staining. EMCL was not detected and was presumably washed away during the fixation and slice preparation. There was a qualitative, but observable decrease in intramyocellular lipid droplet content in the FRSG versus the FC group.

Whole Body Lipid Measurement

Whole body ^{1}H spectroscopy was performed on 28-day rosiglitazone treated rats in order to quantitate whole body lipid content (Fig 6A). The FRSG and the FC groups had similar whole body lipid content (expressed as a percentage of whole body water content) at baseline (48% ± 5% and 44% ± 2%, respectively), but the value was greater in the FRSG group at the end of the treatment period (103% ± 4% *v* 84% ± 6%, respectively, $P < .02$, Fig 6B).

DISCUSSION

In the present study, short-term rosiglitazone treatment in Zucker fatty rats elicited a dramatic reduction ($\downarrow$ ~70%) in tibialis anterior IMCL as assessed noninvasively using ^{1}H NMR spectroscopy. This decrease in IMCL was associated

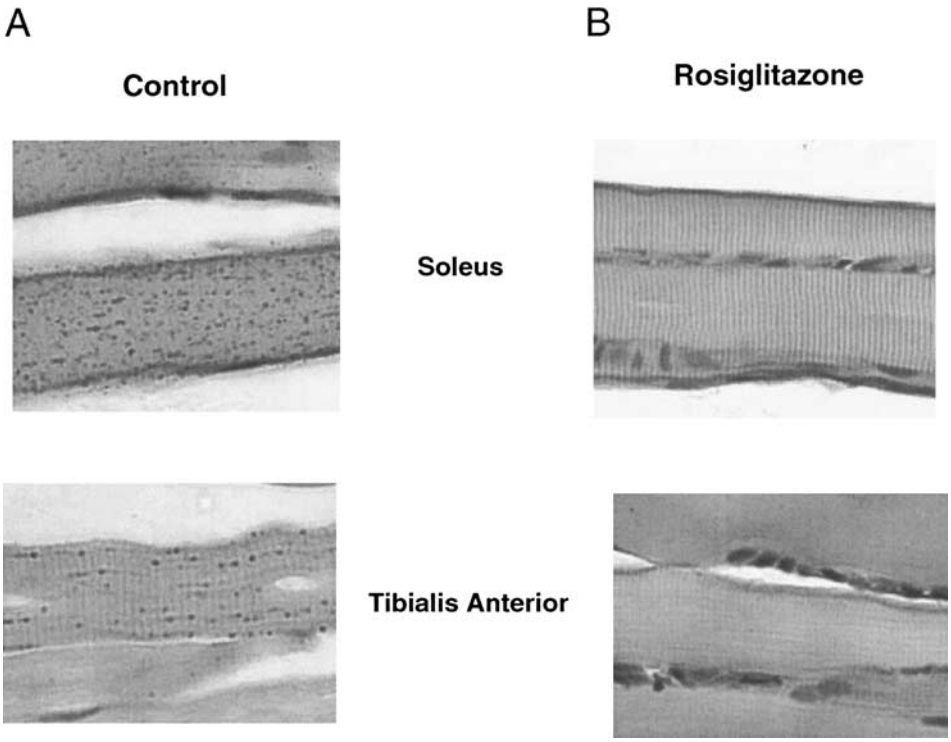

Fig 5. Soleus and tibialis anterior muscle histology. Tissue sections cut parallel to the muscle fiber orientation were stained for lipid content with Oil red O. Qualitative differences in IMCL content in soleus (upper panel) and tibialis anterior (lower panel) muscle in FC (A) and FRSG (B) groups following 7-day rosiglitazone treatment can be observed. The images represent regions of tissue with significant lipid staining. EMCL was not detected and was presumably washed away during the fixation and slice preparation.

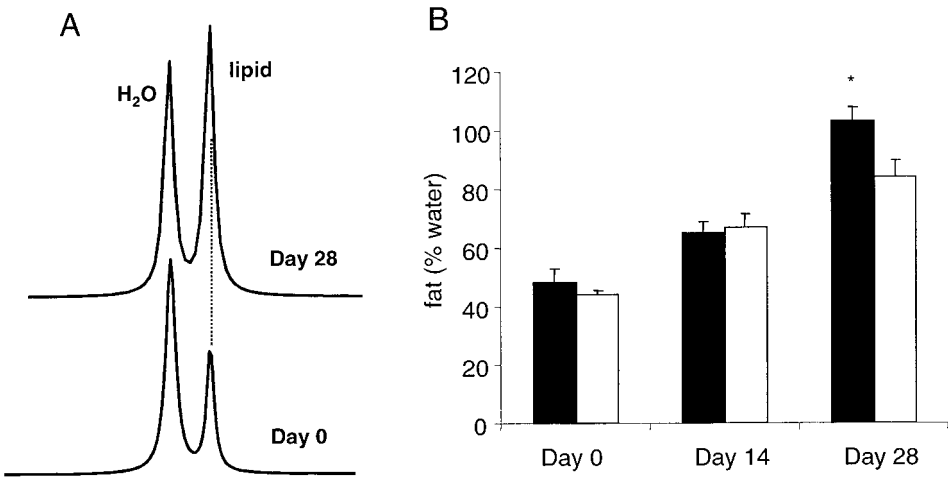

Fig 6. Whole body lipid measurement. Whole body ^{1}H NMR spectroscopy was performed on 28-day rosiglitazone-treated rats in order to quantitate whole body lipid content (A). Whole body lipid spectra were generated using a 10-cm diameter whole body ^{1}H resonator and a single 90-degree hard pulse. (B) Whole body lipid content (expressed as a percentage of whole body water content) in FRSG (■, n = 5) and FC (□, n = 5) groups. Data are reported as mean ± SEM. *P < .02 *v* FC group.

with an increase in insulin-stimulated GIR. In contrast, whole body lipid content was increased following 4-week rosiglitazone treatment.

The relationship between dyslipidemia and insulin resistance in animal[12-14] and man[3-5,11] is well documented. Because skeletal muscle tissue accounts for the majority of whole body insulin-stimulated glucose disposal in man,[33] significant interest in examining the relationship between skeletal muscle lipid metabolite concentrations and insulin sensitivity exists. A negative correlation between skeletal muscle TG content and insulin sensitivity has been established using traditional tissue biopsy[34] and noninvasive NMR spectroscopic[18-21] techniques in humans. Having the ability to discriminate IMCL from EMCL pools, noninvasive NMR spectroscopy represents a powerful analytical tool that can be used to measure the metabolically active intramyocellular lipid. Using NMR, a negative correlation between IMCL and insulin sensitivity was established following chronic, elevated dietary lipid uptake.[22] When plasma FFA is acutely elevated in humans, there is a well characterized time lag of approximately 4 hours before insulin-stimulated glucose disposal begins to diminish.[11,24] To further characterize this phenomenon, soleus and tibialis anterior IMCL in man was shown to accumulate in a time-dependent manner during 5 hours of plasma FFA elevation and euglycemic-hyperinsulinemic clamp.[23] It appears that elevation of systemic plasma FFA alone (− insulin) is sufficient to induce an approximate 30% increase in human IMCL after only 4 hours.[24]

Rosiglitazone is a selective activator of the PPAR-γ receptor. TZDs, including rosiglitazone, have been shown to improve glycemic control in animal models of type 2 diabetes by reducing insulin resistance in muscle,[16,25] liver,[16,25] and adipose tissue.[28,35] Because the molecular target for these agents, PPAR-γ, is primarily expressed in adipose tissue, increases in peripheral tissue insulin sensitivity may be secondary to decreases in systemic TG and FFA supply.[16,25,26] Reduced lipid supply to skeletal muscle would arise from PPAR-γ activation in fat. Almost 40 years ago, Randle et al demonstrated that FFA could directly compete with glucose for oxidation and thereby inhibit glucose uptake via the "glucose–fatty acid cycle."[10] It has also recently been shown that elevated plasma FFA availability is associated with an increase in intramuscular long chain acyl-CoA and diacylglycerol .[7,16,36] Diacylglycerol accumulation has been linked to PKC-activated inhibition of cellular glucose uptake.[7,8] Other groups also have observed a down-regulation of insulin signaling when FFA levels are elevated.[5,6] The hypothesis that TZDs indirectly increase insulin sensitivity in muscle is strengthened by virtue that there was no increase in glucose tolerance in rosiglitazone-treated A-ZIP/F-1 mice, which lack white adipose tissue.[37]

Oakes et al. examined the effect of rosiglitazone on local muscle supply of TG and diacylglycerol in high-fat–fed rats.[16] Although they did not measure a significant difference in muscle TG content, diacylaglycerol was significantly reduced and correlated with improved insulin sensitivity in rosiglitazone-treated rats. They argued that the parallel effects of diet and rosiglitazone treatment on insulin resistance and muscle acylglyceride levels support the involvement of local lipid oversupply in the generation of muscle insulin resistance. In our study as well, there were no differences in tibialis anterior TG content following 7-day rosiglitazone treatment as assessed by biochemical assay. However, when extrapolating absolute IMCL TG content using the NMR derived IMCL/EMCL ratio, IMCL was significantly lower in the FRSG group ($\downarrow$ 70%). This result underscores the utility of in vivo NMR spectroscopy, which differentiates between IMCL and EMCL pools. In a separate study, Oakes et al went on to show that rosiglitazone corrected for hypertriglyceridemia in obese Zucker rats by increasing plasma TG clearance and decreasing hepatic TG production.[30] These data suggest possibly that the static muscle TG level is not as important as its turnover in modulating insulin sensitivity. However, muscle TG content was reduced following treatment with other TZDs in lean[38] and insulin-resistant rodent models,[15,39] and this reduction correlated with increased insulin sensitivity.[15,39] The fact that muscle TG appears to positively correlate with diacylglycerol[16] or fatty acyl-CoAs[15] may justify its use as a marker for insulin sensitivity.

These results as well as our data differ from that of a recent study examining the effects of 3-month rosiglitazone treatment on IMCL and EMCL content in soleus muscle in type 2 diabetics using ^{1}H NMR spectroscopy.[40] Although whole body

insulin sensitivity was increased as assessed using a 2-step euglycemic-hyperinsulinemic clamp, IMCL did not change while EMCL increased 39%. The authors suggested that fatty acyl-CoA may be a more relevant intramyocellular marker that could be correlated with insulin sensitivity. Indeed, it has previously been shown that the metabolically active long-chain acyl-CoAs were reduced in muscle of high-fat–fed rats following 2 weeks of pioglitazone treatment, and this reduction indirectly correlated with the glucose metabolic index.[15] One concern with using IMCL content as an index for insulin sensitivity in humans is that the negative correlation between IMCL and insulin sensitivity may not be valid unless subjects are carefully screened with regard to level of fitness. Recently it was shown that resting IMCL content in tibialis anterior muscle was 71% higher in trained versus untrained subjects.[41] Additionally, species differences may in part be responsible for the differences observed between human and rodent studies. In general, Zucker fatty rats have higher skeletal muscle TG content than in humans. The tibialis anterior muscle TG content we measured biochemically (7.7 μmol/g) is approximately 4-fold greater than in healthy, non-obese subjects (1.6 μmol/g).[42] However, the ratio of gastocnemius TG content in Zucker diabetic fatty versus lean rats,[39] and in Wistar rats fed a high-fat versus starch diet,[16] or soleus IMCL content in obese versus lean humans is comparable ($\sim$2-3).[43]

PPAR-γ activation promotes pre-adipocyte differentiation,[44] increases glucose metabolism and the capacity for adipogenesis,[28,35] and decreases lipolysis.[16,25,26,30,36] The fact that whole body lipid increased following 4-week rosiglitazone treatment in Zucker fatty rats in our study supports the notion that TZDs induce adipogenesis. However, it is interesting to note that the time required for a reduction in IMCL (days) was much shorter than the time required for an increase in whole body lipid to be observed (4 weeks) in our study. This phenomenon may be indicative of a tight homeostatic control between lipolysis and whole body lipid turnover following PPAR-γ activation, such that low circulating lipid metabolites are present while lipid oxidation rates are maintained.

In summary, there is a rapid (days) and pronounced reduction ($\downarrow$ $\sim$70%) in IMCL content in tibialis anterior muscle following rosiglitazone treatment. Additionally, the increase in whole body lipid in the FRSG group suggests that there is increased adipocyte lipid storage following long-term rosiglitazone treatment. These results support the hypothesis that rosiglitazone indirectly increases peripheral insulin sensitivity by decreasing adipocyte lipolysis, thereby lowering the IMCL content.

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