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Rosiglitazone increases fatty acid Δ9-desaturation and decreases elongase activity index in human skeletal muscle in vivo

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ABSTRACT

The ratio of unsaturated to saturated long-chain fatty acids (LC-FAs) in skeletal muscle has been associated with insulin resistance. Some animal data suggest a modulatory effect of peroxisome proliferator receptor γ (PPARγ) stimulation on stearoyl-CoA desaturase 1 (SCD1) and LC-FA composition in skeletal muscle, but human data are rare. We here investigate whether treatment with a PPARγ agonist affects myocellular SCD1 expression and modulates the intramyocellular fatty acid profile in individuals with impaired glucose tolerance. Muscle biopsies and hyperinsulinemic-euglycemic clamps were performed in 7 men before and after 8 weeks of rosiglitazone treatment. Intramyocellular saturated, monounsaturated, and polyunsaturated intramuscular fatty acid profiles were measured by gas chromatography. Effects on SCD1 messenger RNA expression were analyzed in C2C12 cells and in human biopsies before and after rosiglitazone treatment. As expected, treatment with the PPARγ activator rosiglitazone improved insulin sensitivity in humans. Myocellular SCD1 messenger RNA expression was increased in human biopsies and C2C12 cells. Although the total content of myocellular LC-FA was unchanged, a relative shift from saturated LC-FAs to unsaturated LC-FAs was observed in human biopsies. Particularly, the amount of stearate was reduced, whereas the amounts of palmitoleate as well as oleate and vaccenate were increased, after rosiglitazone therapy. These changes resulted in an

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increased fatty acid Δ9-desaturation index (16:1/16:0 and 18:1/18:0) in skeletal muscle and a decreased elongase activity index (18:0/16:0). The PPARγ associated phenotypes may be partially explained by an increased Δ9-desaturation and a decreased elongase activity of skeletal muscle.

1. Introduction

Insulin resistance of the skeletal muscle is a characteristic of obesity and precedes the development of type 2 diabetes mellitus. Several data strongly suggest that accumulation of intramuscular triacylglycerols (IMTGs) contributes to the development of insulin resistance (reviewed by Kelley et al [1] and by Krssak and Roden [2]). However, trained athletes also display high IMTG concentrations (reviewed by Kelley et al [1]) despite preserved insulin sensitivity [3]. The molecular mechanisms linking IMTG accumulation and impaired insulin sensitivity have not yet been fully elucidated, although this metabolic paradox indicates that the total amount of IMTGs might not directly impair insulin action. Various studies demonstrated that specific lipid intermediates, such as the pattern of intracellular saturated and unsaturated fatty acids, may link to the insulin signaling cascade [4-6]. Thus, feeding of rats with saturated fatty acids and exposing cultured human muscle cells or myotubes to the saturated fatty acids palmitate and stearate reduced insulin-stimulated glucose uptake [7-9]. Similar effects were also observed in humans [10]. In accordance with such an assumption, increased intracellular amounts of saturated fatty acids in skeletal muscle were related to insulin resistance [11,12], whereas polyunsaturated long-chain fatty acids (LC-FAs) were associated with improved insulin sensitivity [13]. Moreover, a decrease of unsaturated intramyocellular lipids was recently detected by nuclear magnetic spectroscopy technique in obesity, a state usually characterized by increased insulin resistance [14]. Thus, a considerable body of evidence suggests that the relation of myocellular saturated and unsaturated LC-FAs may substantially contribute to the development of insulin resistance. Stearoyl-CoA desaturase 1 (SCD1) is the rate-limiting enzyme responsible for the conversion of the saturated fatty acids palmitate (16:0) and stearate (18:0) to the unsaturated fatty acids palmitoleate (16:1) and oleate (18:1), respectively. Myocellular SCD1 activity might therefore protect against free FA–induced insulin resistance.

In slight contrast to the mentioned results, SCD1 knockout mice were protected from insulin resistance [15]. Furthermore, increased SCD1 messenger RNA (mRNA) expression and fatty acid Δ9-desaturase index (18:1/18:0) were found in rectus abdominus muscle of extremely obese subjects with insulin resistance [16]; and diet-induced weight loss was accompanied by a reduction of muscle SCD1 protein levels [17]. Considering these controversial data, the role of SCD1 and Δ9-desaturase activity remains unclear in humans.

A pharmacological intervention affecting insulin sensitivity may help to further elucidate this relation. Thiazolidinediones (TZDs), which stimulate peroxisome proliferator receptor γ (PPARγ), are known to improve muscular insulin sensitivity [18]. Even if the exact mechanism beyond those improving effects is not completely understood, various animal data suggest that the activation of PPARγ may also modify intracellular lipid metabolism and the profile of intramyocellular LC-FAs [19,20]. In fact, muscle-specific PPARγ deletion did not alter the total IMTG content [21], whereas the relative amount of intramuscular monounsaturated FAs was increased after PPARγ activation using TZDs [19]. Comparably, rosiglitazone treatment resulted in an increased muscular SCD1 expression in Zucker diabetic fatty rats and mice [22,23], which was recently also confirmed in humans using a randomized controlled trial comparing the effect of pioglitazone and metformin in subjects with impaired glucose tolerance (IGT) [24]. As concomitant changes in myocellular fatty acid composition were not evaluated, it remained unclear whether the effect on SCD1 indeed results in a PPARγ-dependent modification of intramyocellular LC-FA pattern in humans. Several other enzymes, like elongase, are strongly involved in the regulation of intracellular lipid pattern. Interestingly, mice with impaired elongase activity are shown to become obese. However, they are protected from the development of insulin resistance, an effect that is also found during TZD treatment [25]. Thus, we aimed to analyze whether comparable mechanisms might be involved in the regulation of myocellular lipid pattern by TZDs.

Finally, transcription of elongase and SCD1 is regulated by sterol regulatory element-binding protein 1 (SREBP-1) [1,26]. Sterol regulatory element-binding protein 1 increases gene transcription of SCD1 and elongase and favors fatty acid biosynthesis [27,28]. Glucose and insulin modify SREBP-1 expression in animal models [29,30], also providing a potential mechanism linking SREBP-1 to TZD-induced changes of fatty acid composition.

We here evaluated the effects of the PPARγ agonist rosiglitazone on myocellular SCD1 mRNA expression as well as lipid composition in humans in vivo. Modification of SCD1 expression was confirmed in mouse C2C12 myotubes to dissociate direct from indirect (eg, by changes of insulin or glucose) effects.

2. Methods

2.1. *Participants*

Seven male volunteers with IGT were investigated. The baseline anthropometric and metabolic characteristics of the participants have been demonstrated previously (Table 1) [31]. Body weights were stable for at least 2 months before the study. All participants were initially screened for any systemic disease or biochemical evidence of impaired hepatic or renal function. Treatment with insulin, orally taken antidiabetic medication, glucocorticoids, or vitamin K antagonists leads to exclusion from this study. Patients with heart failure, impaired hepatic or renal function, anemia, disturbed coagulation, or any other endocrine disorder were excluded from the

Table 1 – The anthropometric and metabolic characteristics of participants before and after rosiglitazone treatment

	Mean ± SEM pretreatment	Mean ± SEM posttreatment	P
Age (y)	59.3 ± 3.0		
BMI (kg/m^2)	29.3 ± 1.6	29.6 ± 1.4	NS
Waist-to-hip ratio	1.00 ± 0.03	1.02 ± 0.02	NS
Cholesterol (mmol/L)	5.0 ± 0.3	5.3 ± 0.3	NS
HDL cholesterol (mmol/L)	1.4 ± 0.1	1.4 ± 0.0	NS
LDL cholesterol (mmol/L)	2.8 ± 0.2	3.2 ± 0.3	NS
Triglycerides (mmol/L)	1.6 ± 0.3	1.6 ± 0.2	NS
NEFA (mmol/L)	0.41 (0.1)	0.25 (0.3)	<.05
CRP (mg/L)	1.8 (8.0)	0.6 (2.5)	<.05
Creatinine (μmol/L)	79.8 (7.9)	86.7 (7.9)	.063
Fasting insulin (mU/L)	5.7 (6.7)	5.0 (2.7)	NS
2-h insulin (mU/L)	101.8 (87.3)	36.5 (14.3	<.05
Fasting glucose (mg/dL)	95.8 ± 2.6	93.8 ± 1.8	NS
2-h glucose (mg/dL)	168.7 ± 7.6	139.7 ± 10.0	<.05
M-value (mg·kg^{-1}·min^{-1})	3.89 ± 0.68	5.73 ± 0.45	<.05
ISI_{Clamp} (mg·kg^{-1}·min^{-1}/[mU·L^{-1}])	0.07 ± 0.01	0.11 ± 0.01	<.01
Systolic blood pressure (mm Hg)	149 ± 6	138 ± 5	.095
Diastolic blood pressure (mm Hg)	86 ± 3	82 ± 3	NS

Data were compared by paired Student *t* test for normally distributed data and Wilcoxon test for skewed data. Results are expressed as mean ± SEM or median with interquartile range as appropriate.

study. Informed written consent was obtained from all subjects after explanation of the nature, purpose, and potential risks of the studies. The study protocol was approved by the Institutional Review Board of the Charité Medical School, Campus Benjamin Franklin.

2.2. Study design

Each subject was studied with a 3-day protocol. To avoid interactions between the study procedures, the study was performed at intervals of at least 2 days. Following a 10-hour overnight fast, all patients were investigated in the clinical research center at the Charité Medical School, Campus Benjamin Franklin, at 8:00 AM. At 9:00 AM, an oral glucose tolerance test with 75 g glucose was performed. On a different day, a hyperinsulinemic-euglycemic clamp was performed. At the third day, a muscle biopsy was obtained from the gastrocnemius muscle. After baseline characterization, the participants started their treatment with rosiglitazone (GlaxoSmithKline, München, Germany). After 8 weeks on rosiglitazone therapy (2 × 8 mg/d), participants were readmitted and the same 3-day protocol was performed. Because nutritional changes may affect myocellular fatty acid composition, the participants were instructed to retain their individual nutritional behavior during this trial.

2.3. Hyperinsulinemic-euglycemic clamp

The hyperinsulinemic-euglycemic clamp was performed as described previously [31]. In brief, 40 mIU·m^{-2}·min^{-1} human insulin (Actrapid; Novo Nordisk, Bagsvaard, Denmark) and a variable infusion of 10% glucose (Serag Wiessner, Naila, Germany) were used; and capillary glucose concentration was monitored every 5 minutes and was maintained between 4.0 and 4.9 mmol/L via variation of the glucose infusion rate. Insulin sensitivity was assessed as *M*-value and was calculated by dividing the average glucose infusion rate (milligrams glucose per minute) during the steady state of the clamp by the body weight. The insulin sensitivity index (ISI_{Clamp}) was calculated as ratio of glucose metabolized during the steady-state period (*M*-value) to a mean serum insulin concentration (milliunits per liter) in this period in the euglycemic clamp. Blood samples were collected before the clamp and at least 2 hours after starting the clamp during steady-state conditions. Blood samples were centrifuged, and plasma and serum samples were frozen immediately at –80°C.

2.4. Muscle biopsies

Muscle biopsies were taken from the gastrocnemius muscle. The skin was anesthetized with 1% lidocaine without epinephrine. Subsequently, a skin incision (3-4 mm) was made; and a 12-gauge biopsy-cut (CR Bard, Karlsruhe, Germany) needle was used to obtain muscle biopsies from the gastrocnemius muscle. The muscle samples were snap-frozen in liquid nitrogen and stored at –80°C until further analysis.

2.5. Laboratory tests

Capillary blood glucose was measured during hyperinsulinemic-euglycemic clamp every 5 minutes and during oral glucose tolerance test every 30 minutes using the glucose oxidase method on a Dr Müller Super GL, Freital, Germany. Insulin was measured in plasma by enzyme-linked immunosorbent assay (DRG, Marburg, Germany). Interassay coefficient of variation (CV) was 12%, and intraassay CV was 8%. Nonesterified fatty acids (NEFAs) were quantified in serum using a commercially available calorimetric assay (NEFA C; Wako, Neuss, Germany) performed on Cobas Mira (Roche, Basle, Switzerland) (interassay CV, 4.7%; intraassay CV, <5%). Potassium, sodium, serum creatinine, triglycerides, cholesterol, high-density lipoprotein (HDL) cholesterol, protein, C-reactive protein (CRP), and urea were measured using standard laboratory methods. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula [32].

2.6. Fatty acid composition

Muscle samples were extracted, and the lipid fatty acids were transmethylated to fatty acid methyl esters for gas chromatography analysis as described by Larson and Graham [33]. Briefly, muscle samples were ground in a ball-mill (Retsch, Castleford, UK) with acid-washed glass beads in 2 × 3-minute pulses with 200 μL acyl-CoA extraction buffer containing tripentadecanoin internal standard. The lipid fraction was removed with water-saturated petroleum ether, dried down under vacuum, and reacted in sealed tubes with 0.5 mL 1 N HCl in methanol + 0.2 mL hexane for 2 hours at 85°C. After cooling to room temperature, the tubes were opened and partitioned into 2 layers by the addition of 0.25 mL 0.9% (wt/

vol) KCl. The hexane layer was then analyzed for fatty acid methyl ester (FAME) content by gas chromatography (GC Ultra; Thermo Finnigan, Hemel Hempstead, UK). Samples (1 μL) were injected in split mode using H_2 as carrier gas, and FAMEs were separated on a 10-m × 0.1-mm ID BPX-70 column (SGE, Loughborough, UK). The FAMEs were detected with a flame ionization detector and identified and quantified against a 37-FAME standard mix (Supelco, Loughborough, UK). The total LC-FA content was calculated as the sum of the major LC-FA species, namely, palmitate (16:0), palmitoleate (16:1), stearate (18:0), oleate (18:1 n-9), vaccenate (18:1 n-7), linoleate (18:2), linolenate (18:3 n-3), γ-linolenate (18:3 n-6), dihomo-γ-linolenate (20:3 n-6), and arachidonate (20:4 n-6). Furthermore, the contents of the saturated, monounsaturated, and polyunsaturated LC-FAs were calculated as the sum of palmitate (16:0) and stearate (18:0); palmitoleate (16:1), oleate (18:1 n-9), and vaccenate (18:1 n-7); linoleate (18:2), linolenate (18:3 n-3), γ-linolenate (18:3 n-6), dihomo-γ-linolenate (20:3 n-6), and arachidonate (20:4 n-6), respectively.

Furthermore, the ratios of unsaturated FAs to total LC-FAs, monounsaturated FAs to total LC-FAs, and polyunsaturated FAs to total LC-FAs were calculated. In addition, the LC-FA Δ9-desaturation activity index (SCD) (16:1/16:0 and 18:1/18:0) as described by Hulver and colleagues [16] was determined. The Δ6-desaturation (18:3/18:2) and the Δ5-desaturation (20:4/20:3) activity index of LC-FA was calculated as previously described [34]. The elongase activity index of FAs was assessed according to Pan and colleagues (18:0/16:0) [35].

2.7. Cell culture experiments

The C2C12 myoblasts (CRL-1772; ATCC, Manassas, VA) were cultured in Dulbecco modified Eagle medium with 10% fetal bovine serum at 37°C in an atmosphere of 5% CO_2. After confluence, C2C12 myoblast cells spontaneously differentiated into mature myotubes when serum concentration was reduced to 2% by replacing fetal bovine serum with horse serum. At day 6 of differentiation, human myotubes were incubated with serum-free medium (Biochrom, Berlin, Germany) (control) or with serum-free medium supplemented with 1 μmol/L rosiglitazone (Calbiochem, Merck KGaA, Darmstadt, Germany) for 20 hours. All in vitro experiments were performed using at least 5 replicates per treatment.

2.8. Tissue preparation and real-time quantitative polymerase chain reaction

Frozen muscle tissue was homogenized and total RNA from human muscle tissue was isolated according to the manufacturer's instructions of SV Total RNA Isolation (Promega, Mannheim, Germany). RNA samples were stored at –80°C until assayed.

Cells from cell culture experiments were homogenized in Trizol (Invitrogen, Karlsruhe, Germany). After phase separation using chloroform and precipitation using isopropanol, RNA was washed in 75% ethanol, air dried, and then redissolved in RNAse-free water before quantitation.

Complementary DNA (cDNA) synthesis was conducted according to manufacture manual (High Capacity RNA-to-cDNA Kit; Applied Biosystems, Foster City, CA). Samples were analyzed in triplicate with Power SYBR Green PCR Master Mix (Applied Biosystems). Real-time quantitative polymerase chain reaction was performed using an ABI PRISM 7300 System (using SDS 1.4 system software, Applied Biosystems). The expression level of 16s ribosomal protein (16s) was used as an internal control. Primer sequences of analyzed genes are presented in Table S1 of the supplemental data. Cycle threshold values were used to calculate the amount of amplified polymerase chain reaction product in comparison to the housekeeping gene 16s. The relative amounts of each transcript were analyzed using the $2^{-\Delta C(t)}$ method.

2.9. Statistical methods

Statistical calculations were performed using SPSS software version 16 from SPSS (Chicago, IL). Data were compared by paired Student *t* test for normally distributed data and Wilcoxon test for skewed data. Body mass index (BMI), waist-to-hip ratio, cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, 2-hour insulin, fasting glucose, 2-hour glucose, M-value, ISI_{Clamp}, and systolic and diastolic blood pressure as well as the fatty acids 16:0, 16:1, 18:0, 18:1 (18:1 n-7 + 18:1 n-9), 18:1 n-9, 18:2, 18:3 (18:3 n-3 + 18:3 n-6), 18:3 n-3, 20:3 n-6, and 20:4 were normally distributed, whereas NEFA, CRP, creatinine, fasting, 2-hour insulin, FA18:1 n-7, and FA18:3 n-6 were skewed data. Statistical differences between mRNA levels within cell culture experiments were compared using Mann-Whitney test. The correlations between the values were estimated by Pearson correlation test. Multiple linear regression analysis with stepwise variable selection was performed to assess the independent variables. Results were considered to be significant if the 2-sided α was less than .05. Data are presented as mean ± SEM or median with interquartile range as appropriate.

3. Results

3.1. Anthropometric data

No changes in BMI and waist-to-hip ratio and blood pressure were observed during rosiglitazone treatment (Table 1). As previously demonstrated [31], the M-value and the calculated ISI_{Clamp} were increased compared with baseline after 8 weeks on rosiglitazone therapy, suggesting improved insulin sensitivity (Table 1). Correspondingly, glucose and insulin levels 2 hours after oral glucose load were decreased; and reduced CRP levels were observed at the end of rosiglitazone treatment.

3.2. mRNA expression

Rosiglitazone increased SCD1 expression in C2C12 cells by about 30% ($P < .05$), suggesting that direct rather than indirect effects may modify intracellular lipid metabolism (Fig. 1A). In some accordance to the in vitro data, the mRNA expression of SCD1 in skeletal muscle was also increased by rosiglitazone, although the magnitude was greater compared with the in vitro results (Fig. 1B). In contrast, SREBP-1 mRNA expression was not changed by rosiglitazone in human skeletal muscle.

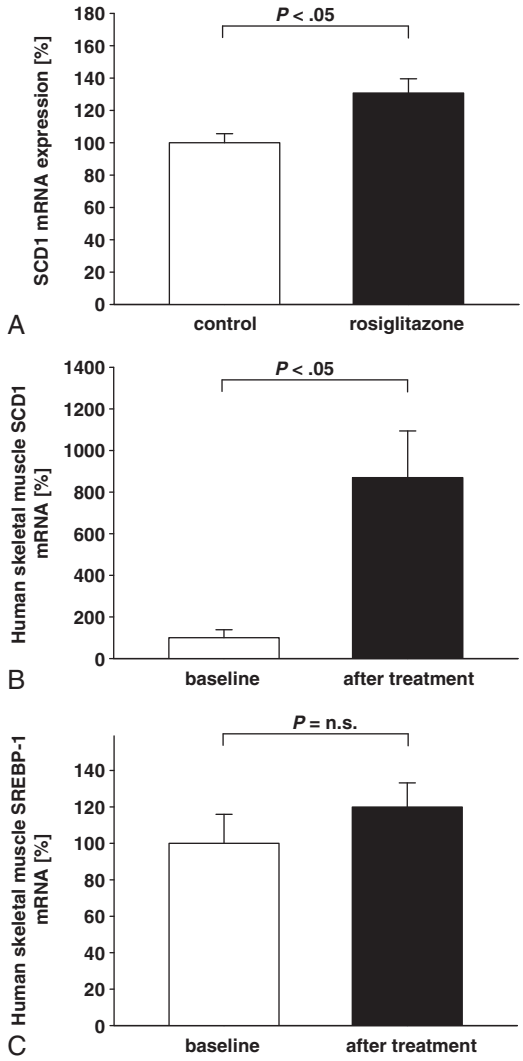

Fig. 1 – Myocellular SCD1 and SREBP-1 mRNA expression. A, Effects of rosiglitazone treatment on SCD1 mRNA expression (percentage) in cultured C2C12 cells (Mann-Whitney test). SCD1 mRNA expression (B) and SREBP-1 mRNA expression (C) in human skeletal muscle (percentage) at baseline and after rosiglitazone treatment (paired *t* test). Results are expressed as means ± SEM.

All human in vivo data were confirmed by using cyclophilin as housekeeping gene (Figure S1 of the supplemental data).

3.3. *Myocellular FAs*

Treatment with rosiglitazone did not change total quantity of saturated (139.6 ± 41.1 vs 147.2 ± 48.4 nmol/mg tissue; *P* = not significant [NS]), unsaturated (274.8 ± 79.5 vs 343.3 ± 117.1 nmol/mg tissue; *P* = NS), or total LC-FAs (414.5 ± 120.4 vs 490.5 ± 165.4 nmol/mg tissue; *P* = NS) compared with baseline. The content of total myocellular FAs did also not differ (462.6 ± 137.6 vs 526.9 ± 176.6 nmol/mg tissue; *P* = NS). However, the relative amount of saturated LC-FAs was decreased by rosiglitazone treatment (Fig. 2A), which was mainly explained by a decline in stearate, whereas no significant change in palmitate was detected (Table 2). Vice versa, a rise in the relative amount of unsaturated LC-FAs was observed after treatment period. Remarkably, the relative increase of LC-FAs was only demonstrated in monounsaturated LC-FAs, whereas the fraction of polyunsaturated LC-FAs was even slightly reduced (Fig. 2B-D). Predominantly palmitoleate, oleate, and vaccenate were increased after rosiglitazone therapy, although the rise in oleate and vaccenate just failed to be significant (Table 2). The reduction in the polyunsaturated LC-FA fraction was induced by a relative decrease of linoleate, dihomo-γ-linolenate, and arachidonate, whereas the relative quantity of linolenate and γ-linolenate was not modified after treatment period (Table 2).

The Δ9-desaturation (SCD dependent) activity index of LC-FAs 16:1/16:0 and 18:1/18:0 was increased in skeletal muscle after treatment period by 44% and 70% (0.27 ± 0.04 vs 0.39 ± 0.03 and 5.09 [5.89] vs 11.94 [1.65]; $P < .05$). Comparable changes were observed in the Δ6-desaturation activity index (18:3/18:2) (0.072 ± 0.006 vs 0.097 ± 0.006; $P < .05$), whereas the Δ5-desaturation activity index (20:4/20:3) (5.29 ± 0.44 vs 4.57 ± 0.61; *P* = NS) was not modified during rosiglitazone treatment. The elongase activity index of FAs was decreased by TZD therapy (Fig. 3).

We next analyzed whether an independent correlation exists between Δ9-desaturation (SCD dependent) activity index of LC-FAs and mRNA expression of SCD1. Given the changes induced by rosiglitazone, we included the data at baseline and after the treatment in the analysis. The SCD1 mRNA expression correlates significantly to the 9-desaturation activity index (18:1/18:1) ($r = 0.650$; $P < .05$), whereas the correlation to 16:1/16:0 marginally failed to be significant ($r = 0.504$; $P = .09$). These correlations were further improved after adjustment for additional confounders (BMI, fasting glucose, fasting insulin, cholesterol, HDL cholesterol, LDL cholesterol, and triacylglycerols) ($r = 0.885$, $P < .05$ and $r = 0.908$, $P < .05$, respectively).

There was a significant correlation of mRNA expression of PPARγ and SCD1 at baseline ($r = 0.862$; $P < .05$), suggesting a substantial role of PPARγ on SCD1 mRNA expression. This was further supported by a multiple linear regression analysis investigating the influence of other contributors of variation of SCD1 gene expression. In this analysis, several metabolic and anthropometric parameters were included (BMI, total cholesterol, LDL cholesterol, HDL cholesterol, triacylglycerols, fasting glucose, fasting insulin, and PPARγ and SREBP1 mRNA expression). Given the modulation induced by rosiglitazone, we again analyzed the baseline and treatment data in this analysis. Stepwise multivariate regression analysis revealed that a model including only fasting glucose and PPARγ mRNA expression as being independently associated with SCD1 could be identified (Table 3), which further supports the assumption of a modulation of SCD1 mRNA expression by PPARγ stimulation.

4. Discussion

We here analyzed the functional effects of PPARγ agonist treatment on intramyocellular lipid profiles in subjects with IGT during an 8-week treatment trial with rosiglitazone. In

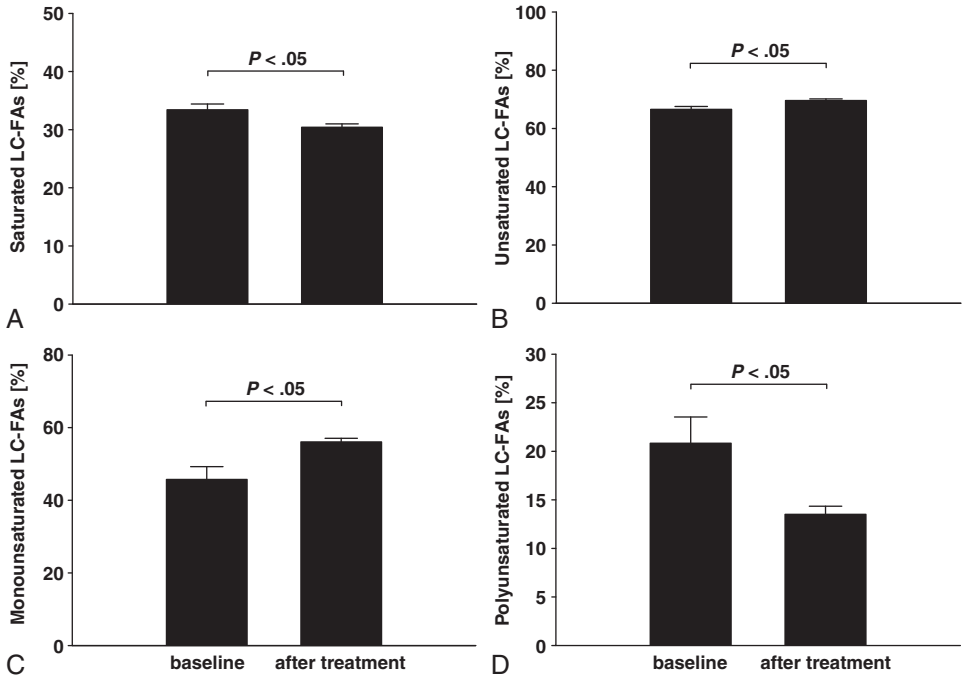

Fig. 2 – Myocellular lipid profile. Saturated LC-FAs (percentage) (A) , unsaturated LC-FAs (percentage) (B), monounsaturated LC-FAs (percentage) (C), and polyunsaturated LC-FAs (percentage) (D) at baseline and after rosiglitazone treatment in human skeletal muscle (paired *t* test). Results are expressed as means ± SEM.

addition to the expected effects on insulin sensitivity [5] and inflammatory parameters like CRP [36], rosiglitazone treatment led to an increase of myocellular SCD1 mRNA expression. This effect was confirmed in murine myotubes, which strongly suggests a direct or at least a cell-specific effect of PPARγ stimulation on SCD1 expression rather than an indirect effect by systemic changes. The increased expression of SCD1 was paralleled by a shift from saturated to unsaturated intramyocellular fatty acids. We specifically observed a Δ9-desaturase–specific pattern of FA modification, supporting the notion that changes of SCD1 expression might be functionally relevant.

Our findings are in agreement with an increased myocellular SCD1 mRNA expression after pioglitazone treatment that was recently described in humans by Yao-Borengasser and colleagues [24]. In this controlled trial, the authors investigated insulin-sensitizing effects of metformin and pioglitazone. Similar to the findings of our own study, a substantial rise in SCD1 mRNA expression was observed in skeletal muscle during pioglitazone treatment. A similar effect was reported by Singh Ahuja et al [22] and Kuda et al [23], who independently demonstrated a TZD-induced increase of intramuscular SCD1 in animals with improved insulin sensitivity. We

Table 2 – Fatty acids composition (percentage) of skeletal muscle at baseline and after rosiglitazone treatment

Fatty acid	Mean ± SEM pretreatment	Mean ± SEM posttreatment	P value
16:0	26.4 ± 0.6	26.6 ± 0.6	NS
16:1	7.0 ± 1.2	10.5 ± 0.9	<.05
18:0	7.0 ± 1.0	3.8 ± 0.3	<.05
18:1 (18:1 n-7 + 18:1 n-9)	38.7 ± 2.6	45.6 ± 0.9	.06
18:1 n-7	2.6 (0.7)	3.2 (0.7)	.06
18:1 n-9	35.9 ± 2.5	42.3 ± 1.0	.07
18:2	13.6 ± 1.3	10.2 ± 0.6	<.05
18:3 (18:3 n-3 + 18:3 n-6)	1.0 ± 0.1	1.0 ± 0.1	NS
18:3 n-3	0.9 ± 0.1	0.9 ± 0.1	NS
18:3 n-6	0.1 (0.1)	0.1 (0.1)	NS
20:3 n-6	1.0 ± 0.3	0.4 ± 0.1	<.05
20:4 n-6	5.3 ± 1.2	1.9 ± 0.3	<.05

Data were compared by paired Student *t* test for normally distributed data and Wilcoxon test for skewed data. Results are expressed as mean ± SEM or median with interquartile range as appropriate.

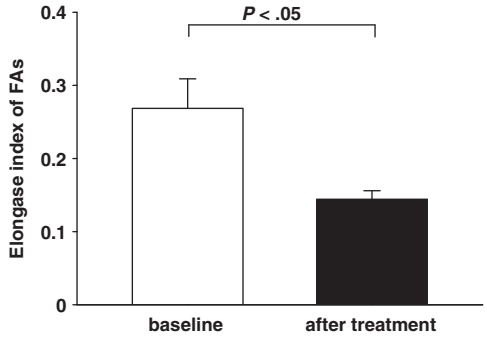

Fig. 3 – Myocellular elongase index. Elongase index of FAs in human skeletal muscle at baseline and after rosiglitazone treatment (paired *t* test). Results are expressed as means ± SEM.

Table 3 – Multiple linear regression showing factors independently associated with SCD1 mRNA expression in human skeletal muscle

Parameter	Correlation	Standardized β	Correlation × standardized β × 100	P
PPARγ mRNA expression	0.767	0.351	26.9	.006
Fasting glucose	0.789	0.443	35.0	.004
Total			61.9	.007

Nonsignificant covariates were subsequently excluded.

here confirm these results by demonstrating an increase of SCD1 expression in human skeletal muscle after 8 weeks of treatment with rosiglitazone. This effect may result from direct PPARγ activation within the skeletal muscle cell; but indirect effects, such as the modification of circulating free fatty acids, cannot be ruled out. Given the significant correlation of human PPARγ and SCD1 mRNA expression in skeletal muscle at baseline and data from our multiple linear regression analysis, some impact of PPARγ on SCD1 mRNA expression can be assumed. Given that the small sample size limits such a multivariate analysis, our results in C2C12 myotubes strongly support a direct or at least muscle-cell–specific effect. The increase of SCD1 was only about 30% in cell culture experiments, whereas much stronger effects were observed in the human trial. The duration of treatment may explain this discrepancy, but indirect effects may also occur.

We next aimed to investigate whether the observed changes of SCD1 expression are functionally relevant. Therefore, changes in LC-FA pattern were analyzed in human skeletal muscle. In accordance to previous human studies assessing the effects of rosiglitazone treatment on myocellular lipid content in patients with type 2 diabetes mellitus [5,37], we did not detect any significant alteration of total quantity of intramuscular fatty acids in subjects with IGT during treatment with TZDs. This is in line with a more recent study demonstrating that pioglitazone did not alter muscle adipose triglyceride lipase expression [38]. Adipose triglyceride lipase represents the key enzyme of hydrolysis of triacylglycerols, which would therefore modulate the total IMTG content. Notably, the quality of FA composition changed substantially during treatment. The increase of unsaturated LC-FAs, especially of the monounsaturated LC-FA fraction, and the decline of saturated LC-FAs are in agreement with an increased Δ9-desaturase activity, which might be explained by the increased myocellular SCD1 expression. The correlation between SCD1 mRNA expression and Δ9-desaturase activity indexes in this study supports this assumption. Previous investigations demonstrated a modulation of SCD1 mRNA expression by PPARγ agonists in human adipose tissue and skeletal muscle [24,34,39]. To the best of our knowledge, this is the first study providing parallel information about directly measured LC-FA composition demonstrating a potential functional relevance of PPARγ-dependent changes in SCD1 mRNA expression. Indeed, changes of intramyocellular lipids were assessed by magnetic resonance spectroscopy in most human trials. Only 2 studies demonstrated decreased intramyocellular lipid content after TZD treatment in diabetic subjects [40] and in individuals with IGT [41] by direct measurement of muscle biopsies. However, these studies did not provide information about the spectrum of myocellular LC-FAs. Therefore, our data provide now the evidence about effects of a PPARγ agonist based on direct measurements of myocellular LC-FAs. Those direct measurements suggest predominant changes of Δ9-desaturase mRNA expression as well as enzyme activity, but also changes of elongase activity.

Interestingly, an increased SCD1 expression and a modification of the fatty acid Δ9-desaturase activity index (18:1/18:0) were also described in skeletal muscle of obese, insulin-resistant subjects [16]. It is known that PPARγ activation also increases body weight. Parts of our strong effects in vivo (in contrast to the existing but moderate effects in vitro) may be explained by obesity-related indirect effects. Although we did not observe significant changes of body weight or composition, respective changes of endogenous circuits may occur. Whether changes of desaturase activity as seen in obesity have effects on insulin sensitivity remains to be elucidated. The observed increase in muscular SCD1 in obese, insulin-resistant subjects may represent a counterbalancing mechanism to protect these subjects from muscular insulin resistance. Further studies are needed to investigate a potential compensatory mechanism.

Human data on elongase activity in skeletal muscle are rare. The indices of muscular elongase activity were previously associated with body fat, whereas no relation to insulin sensitivity was detected in Pima Indians [35]. Given the available reports, there was no evidence of PPARγ-dependent effects on elongase activity in humans. This is now suggested by data presented here demonstrating rosiglitazone-dependent effects on the C18:0/C16:0 ratio, which reflects human elongase activity [35]. Mice deficient in Elovl6, the gene encoding elongase, become obese and develop hepatosteatosis when fed a high-fat diet [25]. Despite this, the mice display a marked protection from hyperinsulinemia, hyperglycemia, and hyperleptinemia, a phenotype that mirrors the phenotype of PPARγ activation. Surprisingly, previous reports demonstrated that troglitazone increases hepatic and muscular elongase activity index in rats [42]. This does not explain the observed phenotype in elongase-deficient mice and is not directly supported by our data. Possible explanations are glitazone-specific effects on elongase activity, which might be realistic because glitazone recruits a different set of nuclear cofactors during PPARγ activation; alternatively, there may be simply a species-specific effect in interactions between PPARγ and elongase. Considering our data and the phenotype of elongase-deficient mice, it is tempting to speculate that PPARγ might affect elongase activity and thereby link to the well-known PPARγ-dependent phenotype.

Finally, we analyzed the mRNA expression of the transcription factor SREBP-1, which is one of the key transcriptional

activators of elongase and SCD1 [1,26]. A rise in SREBP-1 expression would elevate fatty acid synthesis by increased SCD and elongase transcription [27,28]. In agreement with a study of Nadeau and coworkers [43] demonstrating unchanged skeletal muscle SREPB-1 mRNA and protein expression by rosiglitazone in a diabetic animal model, we could not detect any changes of SREBP-1 mRNA expression by rosiglitazone in human skeletal muscle. Thus, SREBP-1 is apparently not involved in the regulation of SCD and elongase by PPARγ agonists.

Even if this study includes results of an in-depth human phenotyping including hyperinsulinemic-euglycemic clamps and human muscle biopsies, some limitations of the present study should be mentioned. This is no placebo-controlled, randomized, crossover trial. Thus, we cannot entirely exclude that parts of the results may be due to changes of environmental factors like increased body weight or modified nutrition during the time course of the experiments. However, body weight was not changed, and the participants were instructed to maintain their diet and physical activity behavior during the trial.

Intraindividually, we detected a comparable amount of total myocellular FA, which may indicate that participants did not, at least substantially, modify their nutrition and physical activity. Nevertheless, we were not able to control all behavioral modalities and other confounding factors. Even if exercise-induced protection against lipid-induced insulin resistance seems not to depend on fat oxidation in skeletal muscle [44], a considerable reduction of whole-body insulin sensitivity can be induced by just a single day of prolonged inactivity [45]. We therefore cannot entirely exclude that those factors may have affected the results. In addition, TZDs are known to recruit a number of nuclear cofactors independent of PPARγ; and rosiglitazone-specific effects on other nuclear cofactors cannot be excluded. Although some of our results were also described in a previous trial using pioglitazone, the comparability may not be consistent with respect to all demonstrated results. Thus, further studies dissecting specific effects of pioglitazone are needed.

Finally, the power of this trial was limited by the sample size of 7 subjects. Although the observed significant changes were detectable, smaller effects on other LC-FA may exist below the detection limit. Therefore, these data should be confirmed in a larger cohort including control subjects.

In summary, the PPARγ agonist rosiglitazone induced an increase of the Δ9-desaturase and elongase activity index in skeletal muscle biopsies, at least partially via direct or at least cell-type–specific effects on rate-limiting enzymes of those processes such as SCD1. Whether those effects contribute to the PPARγ-related phenotype with obesity, but improve insulin sensitivity, remains to be elucidated.

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Conflict of Interest

TB, JA, AA, KB, IG, TL, SD, and AP have nothing to declare. KM and JS received consultant fees from Bristol Myers Squibb. JS has also received lecture and/or consulting fees from Berlin Chemie, MSD, Pfizer, Novo Nordisk, and Sanofi-Aventis.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.metabol.2011.05.018.

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