PROMINENT ROLE OF LIVER IN ELEVATED PLASMA PALMITOLEATE LEVELS IN RESPONSE TO ROSIGLITAZONE IN MICE FED HIGH-FAT DIET

In humans, antidiabetics thiazolidinediones (TZDs) upregulate stearoyl-CoA desaturase 1 (SCD1) gene in adipose tissue and increase plasma levels of SCD1 product palmitoleate, known to enhance muscle insulin sensitivity. Involvement of other tissues in the beneficial effects of TZDs on plasma lipid profile is unclear. In our previous study in mice, in which lipogenesis was suppressed by corn oil-based high-fat (cHF) diet, TZD rosiglitazone induced hepatic Scd1 expression, while liver triacylglycerol content increased, VLDL-triacylglycerol production decreased and plasma lipid profile and whole-body glycemic control improved. Aim of this study was to characterise contribution of liver to changes of plasma lipid profile in response to a 8-week-treatment by rosiglitazone in the cHF diet-fed mice. Rosiglitazone (10 mg/kg diet) upregulated expression of Scd1 in various tissues, with a stronger effect in liver as compared with adipose tissue or skeletal muscle. Rosiglitazone increased content of monounsaturated fatty acids in liver, adipose tissue and plasma, with palmitoleate being the most up-regulated fatty acid. In the liver, enhancement of SCD1 activity and specific enrichment of cholesteryl esters and phosphatidyl cholines with palmitoleate and vaccenate was found, while strong correlations between changes of various liver lipid fractions and total plasma lipids were observed (r=0.74-0.88). Insulin-stimulated glycogen synthesis was increased by rosiglitazone, with a stronger effect in muscle than in liver. Conclusions: changes in plasma lipid profile favouring monounsaturated fatty acids, mainly palmitoleate, due to the upregulation of Scd1 and enhancement of SCD1 activity in the liver, could be involved in the insulin-sensitizing effects of TZDs.

Key words: palmitoleic acid, hepatic lipogenesis, insulin resistance, stearoyl-CoA desaturase, thiazolidinediones

INTRODUCTION

It has been recognized that insulin sensitivity is inversely related to the degree of saturation of body lipids (1). In particular, palmitoleate (16:1 n-7) released from adipose tissue of transgenic mice has been recently shown to enhance muscle insulin action and to suppress its own synthesis in the liver (2). On the other hand, liver-derived lipoproteins represent a major source of plasma lipids, and liver could play a key role in the control of plasma palmitoleate levels and contribute to whole-body lipid profile. However, the consequences of changes in hepatic lipid metabolism with respect to whole-body insulin sensitivity, and especially the mechanisms underlying antidiabetic pharmacotherapy in human patients, remains largely unexplored (1).

Formation of palmitoleate marks lipogenesis and depends on the activity of stearoyl-CoA desaturase (SCD), which converts palmitoyl- and stearoyl-CoA into monounsaturated palmitoleoyl- and oleoyl-CoA, respectively. Hence, the ratio of monounsaturated to saturated fatty acids, especially the palmitoleate/palmitic (16:1/16:0) ratio in plasma or tissues, can be used to estimate SCD activity (3, 4). In mouse, four isoforms of the enzyme (Scd1-4) were identified, while two isoforms (SCD1 and SCD5) exist in humans. In both organisms, high expression of SCD1 gene was detected in lipogenic tissues, i.e. the liver and adipose tissue. Transcription of SCD1 gene could be regulated by several factors, including peroxisome proliferator-activated receptors (PPAR), as reviewed by Paton and Ntambi (3).

Induction of SCD1 by antidiabetic drugs thiazolidinediones (TZDs), specific PPAR-γ agonists, was found in adipose tissue (4, 5) of both type 2 diabetic patients treated by rosiglitazone (4, 5) and in healthy human subjects treated by pioglitazone (6). Moreover, an increase in plasma 16:1/16:0 ratio correlated with improved insulin sensitivity, suggesting a role for the TZD-induced changes in plasma lipid profile in insulin sensitization (4). However, most experiments in rodents and 3T3-L1 adipocytes showed no change, or even a decrease in Scd1 expression/activity due to TZDs, as reviewed by Toyama et al. (7). This discrepancy in the above studies could be explained, at least in part, by a different drive for de novo lipogenesis, which was probably higher in the animals fed standard chow or high carbohydrate diets than in humans consuming a habitual diet (8).
This relatively high lipogenesis in the treated animals, and also in the cultured adipocytes, could mask the stimulatory effect of TZDs. Indeed, up-regulation of Scd1 in liver and muscle by a very low dose of rosiglitazone was observed in our recent study in mice, in which lipogenesis was suppressed by corn oil-based high-fat diet (chHF) (9). In the rosiglitazone-treated mice, liver triacylglycerol (TG) content increased, while hepatic VLDL-TG production, plasma non-esterified fatty acid and triacylglycerol levels decreased, and whole-body insulin sensitivity improved. To characterise complex changes in lipid composition induced by rosiglitazone in the lipogenic tissues of the chHF diet-fed mice, and namely the role of liver in the effect of rosiglitazone on plasma lipid profile, lipidomic analysis of liver, adipose tissue and plasma was performed. Expression of Scd1 in various tissues as well as insulin sensitivity in the liver and skeletal muscle were also assessed. Our results indicate a previously uncharacterized role of the liver in the induction of plasma lipid profile, which could contribute to the antidiabetic action of TZDs.

MATERIAL AND METHODS

Animals, plasma, and tissues

As described before (9), 3-month-old male C57BL/6N mice (Charles River Laboratories, Sulzfeld, Germany) were randomly assigned to chHF diet (lipid content ~35.2% wt/wt, mainly corn oil), or chHF diet supplemented with rosiglitazone (10 mg/kg diet; n=7) with free access to food and water. After 8 weeks of the differential dietary treatment (initial average body weight 27.8±0.7 g and 27.8±0.3 g; final average body weight 44.2±1.1 g and 41.8±1.3 g in the group fed chHF diet and chHF diet supplemented with rosiglitazone, respectively), mice were killed under anaesthesia (75 mg sodium pentobarbital/kg body weight) in ad libitum fed state and EDTA-plasma, liver and epididymal fat were collected. The rate of glycogen synthesis in the liver and skeletal muscle was measured in a separate group of mice subjected to hyperinsulinaemic-euglycaemic clamp (9). Experiments were conducted under the guidelines for the use and care of laboratory animals of the Institute of Physiology and followed the ‘Principles of laboratory animal care’ (NIH publication no. 85-23, revised 1985).

Fatty acid composition

Lipids from plasma, liver, and adipose tissue were extracted with dichloromethane-methanol (2:1, v/v). In the case of liver, cholesteryl ester (CE), TG, phosphatidylcholine (PC), and phosphatidylethanolamine (PE) fractions were separated by preparative thin-layer chromatography and transmethylated as previously described (10). Fatty acid composition was measured using Shimadzu GC-17A gas chromatograph (Tokyo, Japan) equipped with capillary column DB-WAXETR 30 m × 0.32 mm I.D., δ=0.25 μm (J&W Scientific, USA). The oven temperature was programmed from 80 to 130°C at 10°C/min, to 240°C at 2 °C/min, then isothermal 25 min. The injector and detector temperatures were 250 and 270°C, respectively. Hydrogen carrier gas was maintained at a head pressure of 70 kPa and total flow 25 ml/min.

Quantitative RT-PCR

Levels of Scd1 transcript were evaluated in total RNA isolated from the liver, adipose tissue and skeletal muscle, and normalized using elongation factor-1α as before (9).

RESULTS

Differential stimulation of Scd1 expression by rosiglitazone in various tissues

In accordance with our previous study (9), a low dose of rosiglitazone stimulated Scd1 expression in the chHF diet-fed mice in all the tissues analysed, with a much stronger induction observed in the liver than in skeletal muscle and adipose (Fig. 1).

Complex changes of liver lipid composition induced by rosiglitazone

Based on the strong Scd1 upregulation in the liver, we decided to perform a detailed analysis of rosiglitazone-induced changes in liver lipid composition. Fatty acid composition of TG, PE, PC, and CE fractions separated from total liver lipids was analysed (Fig. 2). Palmitoleate was by far the most significantly upregulated fatty acid, showing a striking enrichment in all lipid fractions of rosiglitazone-treated mice, with ~3.0-fold induction in both TG and CE fractions and a smaller increase in phospholipids (fold increase: 3.06±0.33, 3.00±0.43, 2.40±0.18, 1.84±0.13 in TG, CE, PE, and PC fraction, respectively; see Fig. 2). In addition, vaccenic acid (18:1 n-7), a product of palmitoleate elongation, showed a similar but less pronounced response to the rosiglitazone treatment (resulting in a ~1.2-fold increase in plasma levels of this acid). Total contents of saturated, monounsaturated, and polyunsaturated fatty acids in various fractions of hepatic lipids, as well as in total lipids in adipose tissue and plasma are shown in Table 1. Importantly, the amount of monounsaturated fatty acids was increased in all lipid fractions.

![Fig. 1. Effect of rosiglitazone on Scd1 gene expression.](image)

**Fig. 1.** Effect of rosiglitazone on Scd1 gene expression. At 3 months of age, mice were randomly assigned to control chHF diet, or chHF diet supplemented with rosiglitazone. After 8 weeks of treatment, expression of Scd1 was measured in the liver, skeletal muscle and adipose tissue. Black bar, chHF diet; white bar, chHF diet with rosiglitazone (10 mg/kg diet). Data are means ±SE (n=7). *Significantly different from chHF (p<0.05, t-test).
fractions from the liver and in total plasma lipids in response to the rosiglitazone treatment. Next, principal component analysis was performed to reveal the main factor determining the structure of lipid profiles (Fig. 3A-C). The first principal component (y axis) separated mice into two distinct groups, reflecting the rosiglitazone administration and indicating that rosiglitazone induced identifiable changes in global lipid profile (Fig. 3A). If only

Table 1. Fatty acid composition

<table>
<thead>
<tr>
<th>Fatty acid (mol %)</th>
<th>Group</th>
<th>Liver TG</th>
<th>Liver PE</th>
<th>Liver PC</th>
<th>Liver CE</th>
<th>WAT TL</th>
<th>Plasma TL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum SFA</td>
<td>cHF</td>
<td>25.6 ± 0.4</td>
<td>43.3 ± 0.9</td>
<td>45.8 ± 1.2</td>
<td>18.2 ± 0.6</td>
<td>19.3 ± 0.5</td>
<td>30.4 ± 0.3</td>
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<tr>
<td></td>
<td>cHF+Rosi</td>
<td>24.0 ± 0.5*</td>
<td>38.5 ± 0.6*</td>
<td>44.3 ± 0.6</td>
<td>17.7 ± 0.7</td>
<td>19.9 ± 0.4</td>
<td>30.0 ± 0.1</td>
</tr>
<tr>
<td>Sum MUFA</td>
<td>cHF</td>
<td>23.4 ± 0.8</td>
<td>8.2 ± 0.3</td>
<td>5.4 ± 0.2</td>
<td>35.1 ± 1.1</td>
<td>34.7 ± 0.5</td>
<td>9.6 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>cHF+Rosi</td>
<td>35.7 ± 1.8*</td>
<td>9.9 ± 0.1*</td>
<td>7.3 ± 0.2*</td>
<td>44.0 ± 1.2*</td>
<td>35.1 ± 0.1</td>
<td>10.1 ± 1.3*</td>
</tr>
<tr>
<td>Sum n-3 PUFA</td>
<td>cHF</td>
<td>2.4 ± 0.1</td>
<td>11.4 ± 0.7</td>
<td>6.1 ± 0.4</td>
<td>3.2 ± 0.4</td>
<td>1.2 ± 0.2</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>cHF+Rosi</td>
<td>1.3 ± 0.1*</td>
<td>10.7 ± 0.5</td>
<td>5.6 ± 0.9</td>
<td>3.8 ± 0.5</td>
<td>1.5 ± 0.1</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>Sum n-6 PUFA</td>
<td>cHF</td>
<td>48.6 ± 1.1</td>
<td>37.2 ± 0.5</td>
<td>42.7 ± 1.0</td>
<td>43.5 ± 0.7</td>
<td>44.8 ± 0.2</td>
<td>56.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>cHF+Rosi</td>
<td>39.0 ± 1.7*</td>
<td>40.9 ± 0.3*</td>
<td>42.8 ± 0.5</td>
<td>34.5 ± 1.4*</td>
<td>43.5 ± 0.2*</td>
<td>56.8 ± 0.7</td>
</tr>
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</table>

After 8 weeks of treatment, fatty acid composition of various liver lipid fractions (Liver TG, PE, PC, and CE; n=7), total lipid in adipose tissue (WAT TL; n=3) and plasma (Plasma TL; n=3, individual samples pooled from 2-3 mice) was analysed in mice killed in ad libitum fed state - for details see Fig. 2. Fatty acid concentrations (mol %); MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid. *Significantly different from cHF (p<0.05, t-test).
Variables with loading value >0.95 are considered, the first component could be unequivocally interpreted as a shift from polyunsaturated and saturated TG (18:2 n-6; 18:3 n-3; 20:4 n-6; 22:6 n-3; 14:0) to monounsaturated TG (18:1 n-7; 18:1 n-9), CE (16:1 n-7; 18:1 n-7), PE (16:1 n-7; 18:1 n-7) and PC (16:1 n-7) due to rosiglitazone (Fig. 3B). In addition, PC and CE influence on the first component was specific for the rosiglitazone group. These results are consistent with a strong activation of hepatic SCD1 by rosiglitazone and suggest possible importance of fatty acid composition of various lipid fractions for the effects of rosiglitazone. The second principle component (x axis) showed only a weak separation and its underlying mechanism was not apparent.

Enhancement of palmitoleate content in adipose tissue and plasma due to rosiglitazone

Effect of rosiglitazone on lipid composition in adipose tissue was also evaluated. Except for a ~1.8-fold increase in palmitoleate content in total tissue lipids by rosiglitazone, only negligible changes in the abundance of other fatty acids were observed (Fig. 2). Palmitoleate content exhibited also a prominent, ~2.0-fold increase due to rosiglitazone in plasma (Fig. 2). However, levels of several other fatty acids have also changed, and the change of fatty acid profile in plasma lipids and the liver PC and CE fractions (Pearson’s r=0.88, and r=0.83, respectively; p<0.001). Weaker correlations were also found for changes of fatty acid profile in plasma lipids and the liver TG and PE fraction (r=0.74 for both, p<0.001). In contrast, no such correlation between fatty acid profiles in plasma and adipose tissue was found (not shown).

Stimulation of hepatic and muscle glycogen synthesis

Under hyperinsulinaemic conditions, glycogen synthesis in the liver and skeletal muscle was significantly enhanced in mice treated with rosiglitazone (Fig. 4), in accordance with improved insulin sensitivity in these animals (9). The stimulatory effect of rosiglitazone on tissue insulin sensitivity was more pronounced...
in the skeletal muscle compared to the liver (~3.0- and ~2.1-fold increase, respectively).

**DISCUSSION**

Our results demonstrate that in tissues of mice fed Western-type diet rich in corn oil rosiglitazone stimulates SCD1 activity, in conjunction with improved glycemic control, similar to the effects of TZDs found in humans (4–6). We show for the first time that increased palmitoleate synthesis and complex changes of lipid composition in the liver in response to the rosiglitazone treatment could represent the main mechanism underlying the changes in plasma lipid profile, which could be beneficial for the insulin-sensitizing effect of the treatment.

Thiazolidinediones are likely to improve glycemic control mostly by repartitioning fat away from skeletal muscle, as reviewed by Kuda et al. (9). Elevation of hepatic SCD1 by TZDs appears paradoxical given the abundant evidence linking increased lipogenesis in the liver to obesity and insulin resistance. However, in the chHF diet-fed mice treated by a low dose of rosiglitazone, which was sufficient to improve insulin sensitivity, both Scd1 expression and TG content in the liver increased, while liver VLDL-TG production, plasma non-esterified fatty acids and TG decreased (9). That only Scd1 but not fatty acid synthase gene was up-regulated (9) suggests a specific role of increased desaturation activity of hepatic SCD1 in the enhancement of insulin sensitivity by rosiglitazone, independent of de novo lipogenesis. Accordingly, SCD1 determines fatty acid composition of VLDL-TG secreted by the liver (3), while liver-derived lipoproteins represent a major source of plasma lipids. Importantly, also experiments in mice deficient for the long-chain fatty acid elongase Elovl6 documented that hepatic fatty composition, and palmitoleate content in particular, are determinants for insulin sensitivity (11). That our observations in mice could be relevant also to humans is supported by the results of a recent study showing a positive correlation between hepatic SCD1 activity and glycemic control in healthy human subjects; interestingly, this correlation was only found in obese but not in lean subjects (12).

Results of our lipidomic analysis in the liver suggest that specific enrichment of CE and phospholipid fractions by monounsaturated fatty acids, as well as a change of lipid profile from polyunsaturated and saturated TG to monounsaturated TG, CE and phospholipids, may be important for the anti-inflammatory effect of rosiglitazone. Consequently, rosiglitazone-induced changes in plasma lipid composition might affect lipid profiles in the skeletal muscle and contribute to its increased sensitivity to insulin, which was demonstrated by hyperinsulinaemic-euglycaemic clamps before (9). As shown in this study, complex changes of lipid composition in the liver and increased plasma palmitoleate levels in the rosiglitazone-treated mice were associated with enhanced glycogen synthesis under hyperinsulinaemic-euglycaemic conditions in vivo, with a stronger effect in the skeletal muscle than in the liver, thus supporting the functional relationship between increased plasma palmitoleate levels and improved muscle insulin sensitivity. This conclusion is further supported by the recent finding, demonstrating an enhancement of muscle insulin action by TG-palmitoleate infusion in mice (2).

Furthermore, intramuscular fatty acid composition has been previously shown to shift towards a higher proportion of monounsaturated fatty acids in obese rosiglitazone-treated rats (13). Besides rosiglitazone, another TZD pioglitazone, is increasingly used for the treatment of type 2 diabetic patients (14), also upregulated liver Scd1 and increased plasma palmitoleate levels, while improving glycemic control in our experiments in the chHF diet-fed mice in our experiments similar to the effects of rosiglitazone (not shown). Interestingly, in liver of chow diet-fed mice, pioglitazone treatment resulted in the decrease of total TG content, while the ratio of saturated to unsaturated fatty acids decreased only in the free fatty acid fraction, and it was not changed in other lipid fractions (15). Perhaps, the differences between the results of the above study and our experiments could be explained by different composition of the diets used (see the Introduction). Pioglitazone also decreased ceramide concentration in rat skeletal muscle by reducing its de novo synthesis that is dependent on the availability of palmitoyl-CoA, a substrate for SCD1 (16). These results support the notion that the beneficial effect of rosiglitazone on plasma lipid profile could be elicited also by other TZDs, and that this effect contributes to rather than reflects metabolic improvements resulting from the TZD treatment, namely in the muscle. The importance of changes in muscle lipid profile in the insulin-sensitizing effect of TZDs deserves further studies.

We identified the liver as previously uncharacterised tissue with major involvement in the TZD-induced changes of plasma lipid profile in mice fed obesogenic high-fat diet. Provided that hepatic lipid metabolism is similarly affected by TZDs in mice and humans, it is to be inferred that liver has a key role in the modulation of plasma lipid profile in response to TZDs in type 2 diabetic patients and that resulting changes in plasma lipid composition could contribute to improvement of muscle insulin sensitivity. Our results stress the importance of liver as a target in the TZD therapy of human diabetic patients.

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