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SHORT COMMUNICATION

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Changes in adiponectin receptor expression in muscle and adipose tissue of type 2 diabetic patients during rosiglitazone therapy

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Abstract *Aims/hypothesis:* Adiponectin is important in the regulation of insulin sensitivity in man. Its receptors, adipoR1 and R2, have recently been identified, but their expression in adipose tissue and their regulation in response to insulin sensitisation of diabetic patients have never been assessed. We therefore explored the regulation of adipoR1/R2 and adiponectin expression in adipose tissue and skeletal muscle, and of adiponectin plasma concentrations in response to insulin sensitisation by rosiglitazone. *Methods:* Patients with type 2 diabetes were studied in a double-blind, placebo-controlled crossover study, using in vivo arteriovenous techniques of measuring adipose tissue and muscle blood flow, combined with measurement of adipose tissue and skeletal muscle gene expression. *Results:* Rosiglitazone treatment increased adiponectin concentrations by 69%. Skeletal muscle adipoR1 expression was down-regulated from 109.0 (70.1–165.7) (median [interquartile range]) to 82.8 (63.6–89.3) relative units (p=0.04), but adipose tissue adipoR1 expression was up-regulated from 5.3 (4.4–9.4) to 11.2 (4.8–15.3) relative units (p=0.02) by rosiglitazone. In contrast to adipoR1 expression, adipoR2 expression was not altered by rosiglitazone in either of the tissues. The increase in adipose tissue adipoR1 expression with rosiglitazone was associated with increased postprandial triglyceride clearance (r=0.67, p=0.05), and increased fasting fatty acid output (r=0.78, p=0.01) measured in subcutaneous adipose tissue. *Conclusions/interpretation:* AdipoR1 expression is up-regulated in adipose tissue but down-regulated in skeletal muscle by rosiglitazone. These data suggest that adipoR1 plays a role in mediating the effects of adiponectin in specific tissues in relation to insulin sensitisation.

Keywords Adipokines · Human metabolism · Insulin resistance · PPARγ · Rosiglitazone · Thiazoles · Thiazolidinediones

Abbreviations IQR: interquartile range · LPL: lipoprotein lipase · PPARγ: peroxisome proliferator activated receptor-γ

Introduction

Adiponectin is important in mediating insulin sensitivity, and has a number of biological effects. It stimulates fatty acid oxidation in skeletal muscle and liver, decreases hepatic glucose production and increases whole-body glucose uptake [1, 2].

Two adiponectin receptors, adipoR1 and adipoR2, have been described [3]. AdipoR1 is ubiquitously expressed with high levels in skeletal muscle. In contrast, AdipoR2 is more abundantly expressed in the liver.

Thiazolidinedione treatment increases plasma concentrations of adiponectin [4]. In adipocytes, thiazolidinediones directly increase adiponectin gene expression. In fact, a peroxisome proliferator activated receptor response element has been identified in the promoter region of the adiponectin gene [5].

The regulation of adipoR1/R2 expression by peroxisome proliferator activated receptor-γ (PPARγ) is little understood. In vitro exposure of macrophages to rosiglitazone induces the expression of adipoR2, but not of adipoR1 [6].

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The in vivo effects of PPARγ activation on adipoR1/R2 are unknown.

We therefore explored the concomitant regulation of adipoR1/R2 and adiponectin expression in adipose tissue and skeletal muscle, and of adiponectin plasma concentrations in response to rosiglitazone in type 2 diabetic patients. We examined receptor expression in the context of detailed metabolic characterisation of adipose tissue and skeletal muscle metabolism. We also attempted to quantify adiponectin production by human subcutaneous adipose tissue in vivo in humans.

Subjects, materials and methods

Subjects and study design A subgroup of 12 subjects from a previously described study was investigated in a double-blind, placebo-controlled crossover design [7]. Entry criteria included diet-treated type 2 diabetes, age 30–70 years, fasting plasma glucose concentration 7–12 mmol/l, fasting C-peptide concentration ≥0.5 nmol/l and BMI >24 kg/m^2. Subjects were randomised into one of two groups: rosiglitazone 4 mg twice daily for 12 weeks followed by placebo for 12 weeks or vice versa [7]. At the end of each treatment period, patients attended in a faste state for a full-day metabolic investigation. The study was approved by the Oxfordshire Ethics Committee; all subjects gave their written consent.

Metabolic investigations The measurement of arteriovenous differences in fatty acids and triglycerides, and blood flow measurements were made in adipose tissue and skeletal muscle, as described previously [7]. Plasma adiponectin concentrations were measured by sandwich ELISA [8]. Biopsies of vastus lateralis muscle and subcutaneous abdominal adipose tissue were taken 5 h after the ingestion of a standardised meal.

Quantification of adiponectin, adipoR1 and adipoR2 mRNA levels The concentrations of target mRNAs were measured by reverse transcription followed by real-time PCR using a LightCycler (Roche Diagnostics, Meylan, France), as previously described [9]. First-strand cDNAs were first synthesised from 500 ng (skeletal muscle) or 200 ng (adipose tissue) of total RNA in the presence of 100 units of Superscript II (Invitrogen, Eragny, France) using both random hexamers and oligo(dT) primers (Promega, Charbonnières, France). The real-time PCR was performed in a final volume of 20 μl containing 5 μl of a 60-fold dilution of the RT reaction medium, 15 μl of reaction buffer from the FastStart DNA Master SYBR Green kit (Roche Diagnostics) and 10.5 pmol of the specific forward and reverse primers. Primers were selected in order to amplify small fragments and to hybridise in different exons of the target sequences. The following primer sets were used: 5′-CAGAGATGGCACCCC TGGTG-3′ and 5′-TTCACCG ATGTCTCCCTTAG-3′ for adiponectin mRNA; 5′-AAG CACCGGCAGACAAGAGC-3′ and 5′-AAGCACCGGC AGACAAGAGC-3′ for adipoR1 mRNA; and 5′-CTGTGT GCTGGGCATTGCAG-3′ and 5′-CTGTGTGCTGGGCA TTGCAG-3′ for adipoR2 mRNA. For the purpose of quantification, a standard curve was systematically generated with six different amounts (150 to 30,000 molecules/tube) of purified target cDNA cloned in the pGEM plasmid (Promega). Each assay was performed in duplicate and validation of the real-time PCR runs was assessed by evaluation of the melting temperature of the products and by the slope and error obtained with the standard curve. The analyses were performed using the LightCycler software (Roche Diagnostics). Cyclophilin mRNA levels were measured as internal standard and the data were expressed as relative units as a ratio of cyclophilin mRNA concentrations.

Statistical analysis Data were analysed using SPSS v. 10 (SPSS UK, Chertsey, UK) and statistical significance was set at p=0.05. Data are presented as median (interquartile range [IQR]) unless otherwise stated, when they are presented as mean±SEM. Differences between placebo and rosiglitazone treatments were analysed using paired t-tests. Correlations of adiponectin receptor expression with relevant physiological data were assessed using Spearman rank correlation coefficients. Spearman rank correlation coefficients were used to assess correlations between adiponectin receptor expression and physiological data likely to be important in the regulation of fatty acid metabolism.

Table 1 Characteristics of subjects

	Placebo	Rosiglitazone
BMI (kg/m^2)	30.8±1.04	30.83±1.11
Body fat mass (kg)	33.5±2.29	33.9±2.47
HbA_1c (%)	7.52±0.38	7.17±0.26[a]
Fasting glucose (mmol/l)	7.73±0.4	6.87±0.34[a]
Fasting insulin (pmol/l)	99±12	82±11[a]
Haematocrit	0.42±0.01	0.41±0.01
Total cholesterol (mmol/l)	5.22±0.36	5.82±0.37[a]
Triglycerides (mmol/l)	2.02±0.32	2.02±0.33
NEFA (μmol/l)	525±36	618±48
Plasma adiponectin (μg/ml)	5.7 (4–6.7)[b]	9.6 (6.5–14.5)[a,b]
Adipose tissue adiponectin mRNA (relative units)	66.6 (52.4–82.8)[b]	85.6 (67.9–124.6)[a,b]

Values are means±SEM unless otherwise indicated
[a] p≤0.05 vs placebo
[b] Median (IQR)

Results

Metabolic effects of rosiglitazone therapy The in vivo physiological changes, including insulin sensitisation, during the treatment with rosiglitazone have been described elsewhere [7]. The characteristics of the subgroup investigated here are presented in Table 1; they did not differ significantly from the whole cohort previously published in the variables measured or in the responses to rosiglitazone [7]. Notably, this subgroup showed no change in fasting plasma NEFA concentrations with rosiglitazone treatment, in contrast to rodent studies, as highlighted in a previous paper [7].

Changes in adiponectin expression and concentration Rosiglitazone increased arterialised plasma adiponectin concentrations by 69% (from 5.7 [4.0–6.7] μg/ml after placebo to 9.6 [6.5–14.5] μg/ml after rosiglitazone, $p=0.02$). Concomitantly, adipose tissue adiponectin mRNA levels increased by 28.5% with rosiglitazone treatment (from 66.6 [52.4–82.8] relative units after placebo to 85.6 [67.9–124.6] relative units after rosiglitazone, $p=0.005$). The arteriovenous difference of adiponectin across adipose tissue was indistinguishable from zero both in subjects treated with placebo and in those with rosiglitazone treatment, suggesting a low rate of secretion; no difference in the output of adiponectin from adipose tissue was detected with rosiglitazone treatment [from 0.03±0.06 [mean±SEM] to 0.06±0.12 $ng \cdot min^{-1} \cdot 100\ g^{-1}$ tissue, $p=0.29$).

Regulation of the expression of adiponectin receptors by rosiglitazone The mRNA of both adiponectin receptors could be readily quantified in skeletal muscle and in adipose tissue of type 2 diabetic patients (Fig. 1). As shown in Fig. 1, rosiglitazone therapy was associated with a 24% decrease in adipoR1 mRNA levels in skeletal muscle (from 109.0 [70.1–165.7] to 82.8 [63.6–89.3] relative units, $p=0.04$). In contrast to skeletal muscle, adipose tissue adipoR1 mRNA doubled with rosiglitazone (from 5.3 [4.4–9.4] to 11.2 [4.8–15.3] relative units, $p=0.02$). AdipoR2 mRNA expression was not modified by rosiglitazone therapy in skeletal muscle (from 42.8 [33.5–45.1] to 28.6 [24.1–40.1] relative units, $p=0.33$) or in adipose tissue (from 9.0 [6.6–13.4] to 12.1 [9.4–16.0] relative units, $p=0.13$). There was no significant correlation between plasma adiponectin concentrations and adipoR1 or adipoR2 mRNA expression in muscle or adipose tissue (data not shown). Similarly, adipose tissue adipoR1/R2 mRNA expression was unrelated to adipose tissue adiponectin mRNA expression.

To assess whether the changes in adipoR1 gene expression were associated with functional physiological changes, we compared the changes in adipoR1 with the changes in physiological parameters important in energy metabolism, such as triglyceride removal and fatty acid uptake. In adipose tissue, as adipoR1 mRNA concentrations increased, postprandial adipose tissue clearance of triglycerides increased ($r=0.67$, $p=0.05$), as did fasting NEFA release from adipose tissue ($r=0.78$, $p=0.01$). The increase in adipoR1 expression in adipose tissue correlated

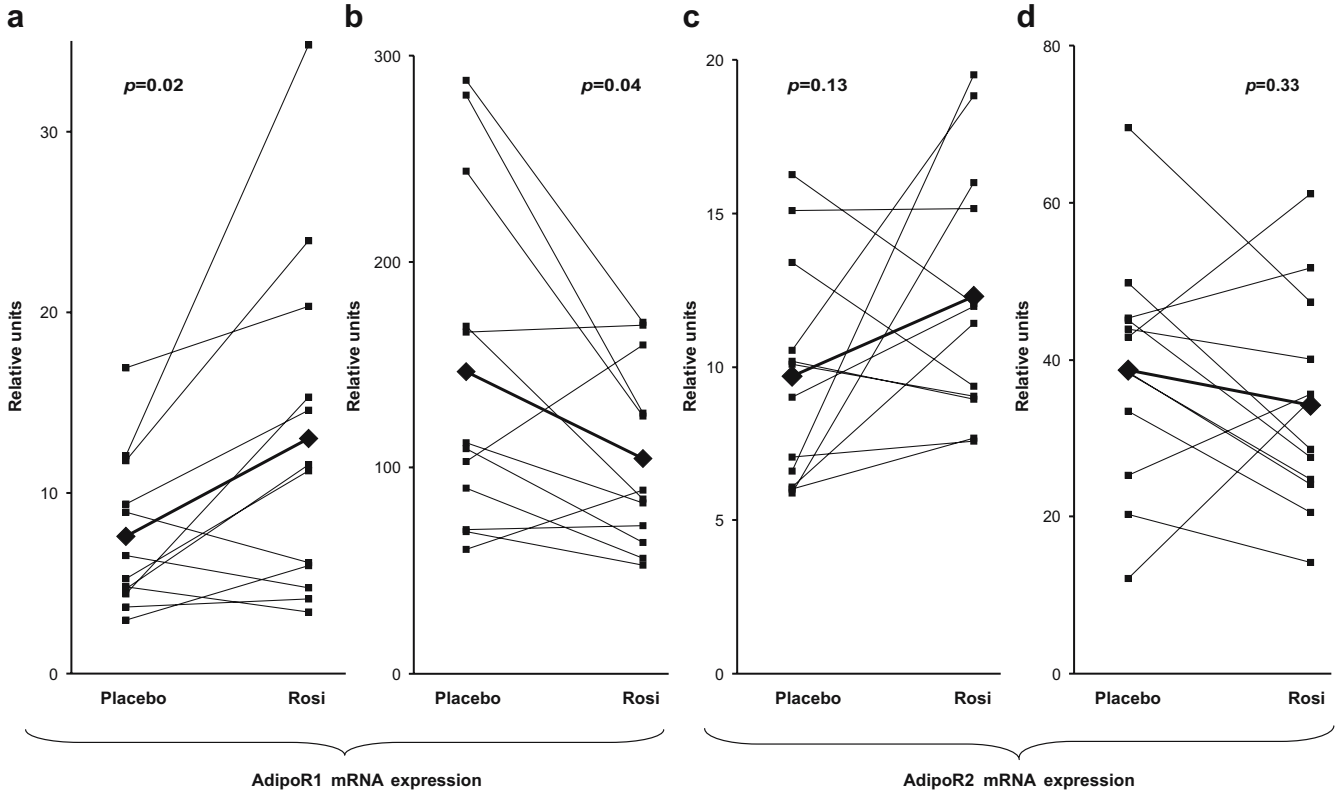

Fig. 1 Adiponectin receptor 1 and 2 mRNA expression in response to rosiglitazone (Rosi) treatment and placebo in adipose tissue (**a**, **c**) and muscle (**b**, **d**). Mean values: *filled diamond*

with the decrease in fasting insulin concentration (r=−0.79, p=0.036).

In skeletal muscle, as adipoR1 mRNA concentrations decreased, fasting and postprandial total fatty acid uptake into muscle increased (r=0.67, p=0.05), but triglyceride removal was unchanged (r=−0.10, p=0.80). The change in expression of adipoR1 was unrelated to the change in muscle glucose uptake.

Discussion

This is one of the first studies to describe the expression of adiponectin receptors, adipoR1 and adipoR2, in human adipose tissue and to examine their in vivo regulation in human skeletal muscle and adipose tissue. We show that rosiglitazone treatment of diabetic subjects regulated adipoR1 expression in different ways in adipose tissue and skeletal muscle: adipoR1 expression is down-regulated in skeletal muscle, but up-regulated in adipose tissue. AdipoR2 expression is not regulated by rosiglitazone.

One effect of the insulin sensitiser rosiglitazone is to reduce insulin concentrations in hyperinsulinaemic subjects. AdipoR1 expression is negatively regulated by insulin [10], which could explain the up-regulation of adipose tissue adipoR1 seen here. Our human in vivo finding of an inverse correlation between changes in plasma insulin concentrations and changes in adipose tissue adipoR1 concentrations supports Tsuchida and colleagues' rodent findings [10]. In skeletal muscle, down-regulation of adipoR1 by rosiglitazone occurs as adiponectin concentrations increase. This appears to conform to the common feedback regulation of hormone–ligand receptor interactions, of which leptin is an example.

What might be the physiological effect of this up-regulation of adipose tissue adipoR1 in the presence of increased plasma adiponectin concentrations? There was an inverse correlation between the change in adipoR1 expression in adipose tissue and in vivo postprandial triglyceride clearance in adipose tissue, reflecting LPL activity. Increased signalling through adipoR1 in adipose tissue could modulate triglyceride removal from the circulation. This, in turn, would promote fat storage in the postprandial state and may participate in the increase in subcutaneous abdominal adipose tissue mass seen with thiazolidinedione treatment. However, the change in adipose tissue adiponectin receptor expression is an effect associated with rosiglitazone treatment, but specific cause–consequence relationships need to be analysed in future. In skeletal muscle, the decrease in adipoR1 by rosiglitazone correlated with fatty acid uptake. This observation apparently conflicts with the proposed role of adipoR1 in the activation of fatty acid utilisation and oxidation [3] but may just reflect adaptation to high circulating adiponectin concentrations induced by rosiglitazone treatment.

Only one recently published study has previously described adipoR1/R2 expression in adipose tissue. Hammarstedt and colleagues treated non-diabetic insulin-resistant subjects with pioglitazone for 3 weeks and took adipose tissue biopsies before and after treatment [11]. In contrast to our findings, their study did not find any change in adipoR1 and R2 expressions in adipose tissue. There were a number of important differences between the studies, which may account for some of the discrepancies. In our study, we used type 2 diabetic subjects, whilst Hammarstedt and colleagues used insulin-resistant non-diabetic subjects. Our placebo-controlled double-blind study looked at long-term treatment with rosiglitazone, a pure PPARγ agonist; Hammerstedt and colleagues used pioglitazone, which may have some PPARα, as well as PPARγ, agonist activity [12]. The duration of treatment differed between the studies: treatment was for 3 months in our study but 3 weeks in the study by Hammarstedt and colleagues. As thiazolidinediones can take up to 3 months to exert their effects, our study would identify chronic adaptive changes with thiazolidinediones, but would not distinguish adaptive changes secondary to improved glucose or lipid metabolism. Therefore the lack of change in adipose tissue adipoR1 expression found by Hammarstedt and colleagues, despite inducing insulin sensitisation, might reflect that adipose tissue adipoR1 expression increases later in the course of treatment with thiazolidinediones.

In this study, we attempted, for the first time, to measure arteriovenous differences of adiponectin across its main site of production, subcutaneous abdominal adipose tissue. Despite the rosiglitazone-induced increase in plasma adiponectin concentrations, as seen in other studies [4], no arteriovenous differences in adiponectin concentrations were detected. One explanation would be that if adiponectin had a long half-life with a low turnover, which would be implicit with a low rate of production, there would be a low rate of production by adipose tissue. The coefficient of variation of a typical ELISA used to measure adiponectin is 2–8%, whilst the arteriovenous difference averaged about 0.5% of the circulating adiponectin concentrations.

In summary, this work provides evidence that the expression of adiponectin receptors is affected by insulin sensitisation with rosiglitazone in human skeletal muscle and adipose tissue. The association between adipoR1 expression in adipose tissue and the clearance of triglyceride may indicate a contribution of adiponectin and its receptors to fat metabolism in vivo in humans.

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Duality of interest

This study was initiated and led by the investigators, who received funds from GlaxoSmithKline (GSK) for the running costs of the study. However, GSK played no role in the design of the study, in the collection and interpretation of the data, or in the writing of the report. The authors have no financial interest in GSK.

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