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The effect of the PPAR γ ligand rosiglitazone on energy balance regulation

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Abstract

Background and aim Fat mass generation requires an energy surplus and the activity of the peroxisome proliferator-activated receptor γ (PPAR γ). We investigated if the PPAR γ ligand rosiglitazone influences substrate usage, energy expenditure (EE) and energy intake (EI) and, thereby, how PPAR γ activity contributes to susceptibility to obesity.

Methods Twenty healthy males (20–29 years) were randomly assigned to receive a placebo ($n = 10$) or rosiglitazone (8 mg/d) ($n = 10$) for seven consecutive days, while staying in a respiration chamber. Food intake was *ad libitum*. Body composition was determined by underwater weighing (day 1) and deuterium dilution (day 1 and 8).

Results Mean (\pm SE) EI was 15.9 ± 0.9 MJ/d in the placebo group and 18.9 ± 1.2 MJ/d in the rosiglitazone group. Mean EE was 11.3 ± 0.3 MJ/d and 12.5 ± 0.5 MJ/d for the placebo and rosiglitazone groups respectively. This resulted in a cumulative positive energy balance (EB) of 32.3 ± 5.1 MJ for placebo and 44.7 ± 6.9 MJ for rosiglitazone. There were no significant differences in EI, EE, and EB between treatments. Both groups did not adjust their fat oxidation to the increased fat intake, but fat oxidation decreased faster in the rosiglitazone group (significantly lower on days 6 and 7). During treatment with rosiglitazone, significantly more fat storage was seen in overweight subjects while this was not the case in the placebo group.

Conclusions Our results suggest a shift in substrate usage during PPAR γ stimulation leading to a preference for fat storage, especially in subjects with a higher BMI. Copyright © 2005 John Wiley & Sons, Ltd.

Keywords peroxisome proliferator-activated receptor γ ; substrate usage; energy expenditure; energy intake; energy storage; humans

Introduction

Obesity develops when energy intake (EI) exceeds energy expenditure (EE) for longer periods. Excess energy is stored in the main energy storing tissue, the adipose tissue. The adipocyte is not only an important mediator of energy metabolism by storing excess energy as fat, but also by secreting adipokines involved in energy metabolism, like leptin, adiponectin and resistin [1]. Fat mass generation requires an energy surplus and the activity of the peroxisome proliferator-activated receptor γ (PPAR γ), a transcription factor that is expressed at high levels in adipose tissue, and at lower levels in the skeletal muscle, liver and heart [2,3]. Dominant-negative PPAR γ mutations lead to partial lipodystrophy [4], while dominant-positive PPAR γ mutations

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are associated with severe obesity [5]. PPAR γ is a key factor in fat metabolism as it is required for adipogenesis, the differentiation of preadipocytes into mature adipocytes, and has an important role in fat storage [6]. PPAR γ is a member of the nuclear hormone receptor family whose transcriptional activity is regulated by the formation of heterodimers and the binding of ligands [7]. Natural ligands include polyunsaturated fatty acids and eicosanoids [8]. Potent synthetic ligands include the anti-diabetic thiazolidinedione (TZD) class of drugs [9]. TZDs are widely and effectively used in the treatment of type 2 diabetes mellitus for their insulin-sensitizing effects [9]. However, treatment with TZD is accompanied by weight gain in rodents [10] and obese, diabetic patients [11]. Though fluid retention is a known side effect of TZD treatment [12], the major part of the weight gain consists of fat [10] which does suggest an effect of PPAR γ on energy balance (EB) regulation as fat gain will only occur in the presence of an energy surplus. Indeed, PPAR γ activation in lean rats, in diet-induced obese rats, and in insulin-resistant fatty Zucker rats resulted in weight gain because of both an increased food intake and an improved feed efficiency [13–15]. On the other hand, pair-feeding of chow-fed rats and dietary obese rats to prevent hyperphagia prevented rosiglitazone-related weight gain in chow-fed rats, though this was not the case with the dietary obese rats [13]. Similar results were observed in humans. Obese, diabetic patients treated with TZD, who reported weight gain following TZD treatment, effectively lost weight with caloric restriction and increased physical activity [11]. These observations emphasize a possible role for PPAR γ in the regulation of EI and expenditure. We hypothesized that stimulation of PPAR γ with the PPAR γ ligand rosiglitazone in healthy, normal to overweight men would induce susceptibility to weight gain by affecting substrate usage, EE and EI.

Subjects and methods

Subjects

Subjects were recruited through noticeboards in the university and in the university hospital. Subjects had to be male, between the age of 18 and 40 years, Caucasian, healthy and have a body mass index (BMI) between 20 and 32 kg/m². They had to be unrestrained eaters, as indicated by the Three-Factor Eating Questionnaire [16] with scores ≤ 9 on Factor 1 (cognitive restraint). Subjects completed a medical questionnaire before entering the study; only subjects in good health were included. Subjects who followed a dietary regime with the aim to lose or gain weight within a year prior to the study were excluded. The study design required that the subjects were not fully informed about the adipogenic effect of the PPAR γ agonist rosiglitazone as this could influence self-selected food and EI and activity-induced EE. Therefore, subjects were told that they would receive either a substance that influences fat metabolism or a non-active

Table 1. Baseline characteristics of the subjects

	Placebo (n = 10)		Rosiglitazone (n = 10)	
	Mean	SE	Mean	SE
Age (years)	22	1	24	1
Height (m)	1.83	0.02	1.84	0.02
Body weight (kg)	80.2	3.5	85.7	3.6
BMI (kg/m ²)	24.0	1.3	25.1	0.7
Body fat (%)	19.6	1.9	20.3	2.4
Cognitive restraint score ^a	5	1	4	1

^aFactor 1 of the three-factor eating questionnaire [16].

substance (placebo) in a double-blind manner (i.e. neither the subject nor the investigator knew what the subject received during the experiment). The study was approved by the Ethics Committee of Maastricht University. All subjects received verbal and written information and signed a written consent form. Twenty men between the age of 20 and 29 years participated in the study. Characteristics of the subjects are shown in Table 1.

Experimental design

Subjects were studied during a stay in a respiration chamber for seven consecutive days for EE and substrate oxidation measurements. Following a double-blind, placebo-controlled design, subjects were randomly assigned to receive either 8 mg/d rosiglitazone (Avandia, GlaxoSmithKline BV, The Netherlands) or a placebo orally. Drugs were dosed twice daily for the total stay of 7 days in the respiration chamber. Subjects were asked to eat *ad libitum* from an excess of food supplied at each mealtime and as snacks. No exercise protocol was imposed, but sleeping during daytime and strenuous physical activity were not allowed. Subjects were woken up between 08:00 and 08:30 h, and they were free to choose their bedtime in the evening. Body composition was determined by underwater weighing and deuterium dilution (day 1) or by deuterium dilution alone (day 8). On the same days, blood samples were taken.

Dietary intake

Meals consisting of typically Dutch food items were provided three times per day, breakfast between 08:30 and 09:00 h, lunch between 12:30 and 13:00 h, and dinner between 18:00 and 18:30 h. Breakfast and lunch consisted of bread, savoury and sweet condiments, fruit, yogurt, milk, fruit juice, instant coffee (decaffeinated) and tea. For dinner, subjects could choose between ready-prepared potato-, pasta- or rice-based meals with only vegetables or with vegetables plus meat or fish. They were allowed two alcoholic consumptions, white or red wine or beer, per day. A wide variety of snacks was continuously available in the chamber in packages that were refreshed every morning. Snacks consisted of savoury items (crisps, nuts), sweet items (chocolate bars,

sweets), various biscuits, fruit (apple, orange, banana), instant soup, fruit juice, instant coffee (decaffeinated) and tea. Meals and snacks were supplied in excess to be eaten *ad libitum*; extra food items were readily available on request. All foods and drinks entering and leaving the respiration chamber were weighed to the nearest gram. Energy content and macronutrient composition of the diets were calculated using the Dutch food composition table (NEVO 1996).

Procedures

Anthropometry and body composition

Measurements were carried out in the morning after voiding and before breakfast. Body weight and height were measured to the nearest 0.01 kg and 0.1 cm respectively. Body mass index (BMI, kg/m²) was calculated as body weight (kg) divided by height (m) squared. Body density was determined by underwater weighing with simultaneous measurement of residual lung volume with the helium dilution technique. Total body water (TBW) was determined with deuterium dilution following the Maastricht protocol [17]. Body composition was calculated from body density and TBW using the three-compartment model of Siri [18].

Energy expenditure

EE was measured in the respiration chamber [19] from oxygen consumption, carbon dioxide production and urinary nitrogen excretion according to the formula of Brouwer [20]. The respiration chamber measured 14 m³ and was furnished with a bed, chair, table, TV, radio, telephone, computer, washbowl and toilet facilities [19].

Substrate oxidation

Carbohydrate (CHO), fat (F) and protein (P) oxidation was calculated from oxygen consumption, carbon dioxide production and urinary nitrogen excretion. As subjects were allowed to have maximally two alcoholic consumptions per day, oxygen consumption and carbon dioxide production on these days were first corrected for alcohol intake, assuming that all alcohol ingested was oxidized, before calculation of CHO, F and P oxidation according to the formula of Brouwer [20].

$$P \text{ oxidation (g/d)} = 6.25 * N$$

$$F \text{ oxidation (g/d)} = 1.718 * VO_2 - 1.718 * VCO_2 - 0.315 * P$$

$$CHO \text{ oxidation (g/d)} = 4.17 * VCO_2 - 2.965 * VO_2 - 0.390 * P$$

with:

N = total nitrogen excreted in urine (g/d)

VO_2 = oxygen consumption (l/d), corrected for alcohol intake

VCO_2 = carbon dioxide production (l/d), corrected for alcohol oxidation

P = protein oxidation (g/d)

24-h urine was collected from the second voiding of the day until the first voiding of the following day. 24-h urines were collected for each of the 7 days in the respiration chamber separately. Urine bottles contained 10 ml H₂SO₄ to prevent nitrogen loss through evaporation. Volume and nitrogen concentration were measured, the latter using a nitrogen analyser (Elemental Analyser, CHN-O-Rapid, Heraeus).

Carbohydrate and fat balances were calculated as intake minus oxidation of each macronutrient. The protein balance was calculated as [N intake – (urinary N + faecal N)] * 6.25, assuming an average daily value of 3 g/d for faecal N loss [21].

Blood parameters

After an overnight fast, blood samples were obtained and mixed with citrate to prevent clotting. Plasma was obtained by centrifugation (4°C, 3000 rpm, 10 min), frozen in liquid nitrogen and stored at –80°C until analysis of concentrations of glucose (hexokinase method, Glucose HK 125 kit, ABX Diagnostics, Montpellier, France), insulin (ELISA, Mercodia, Uppsala, Sweden), leptin (double-antibody RIA, human leptin specific RIA kit, Linco Research Inc., St Charles, USA) and triacylglycerols (TAGs) (Triglycerides liquicolor kit, Instruchemie, Delfzijl, The Netherlands).

Statistical analysis

Results are presented as mean ± standard error (SE). Differences between treatments were analysed with one-way analysis of variance (ANOVA) or the Mann-Whitney U-test (blood parameters). Interaction between treatment and body weight class for fat balance was analysed with an univariate ANOVA with treatment and body weight class as fixed factors. Measurements at baseline and at the end of the experiment were compared with Student's paired *t*-test (two-tailed) or Wilcoxon's paired signed rank test (blood parameters). $P < 0.05$ was considered statistically significant. SPSS 11 for Macintosh (SPSS Inc., Chicago, IL, 2002) was used for the analysis.

Results

Energy intake and expenditure

The mean daily EI was 15.9 ± 0.9 MJ/d for placebo and 18.9 ± 1.2 MJ/d for rosiglitazone, which was not significantly different ($P = 0.06$). Mean daily macronutrient

composition was not significantly different between treatments, except for CHO intake, which was higher in the rosiglitazone group ($P < 0.05$).

Similar results were obtained for EE and macronutrient oxidation. EE was not significantly different between treatments ($P = 0.09$) with mean daily values of 11.3 ± 0.3 MJ/d for placebo and 12.5 ± 0.5 MJ/d for rosiglitazone. Mean daily macronutrient oxidation was not significantly different between treatments, except for CHO oxidation, which was higher in the rosiglitazone group ($P < 0.05$).

Energy balance

Energy balance (EB) was calculated as the difference between EI and EE. Figure 1 shows that cumulative EB increased from day 1 to day 7 on both treatments. By the end of day 7, cumulative EB was 32.3 ± 5.1 MJ for placebo and 44.7 ± 6.9 MJ for rosiglitazone. This differed significantly from zero on both treatments ($P < 0.001$), but was not significantly different between treatments ($P = 0.16$). The positive EB was reflected in changes in body weight. Body weight increased by 1.39 ± 0.37 kg ($P < 0.01$) in the placebo group and by 2.54 ± 0.53 kg ($P = 0.001$) in the rosiglitazone group. Changes in body weight did not differ significantly between treatments ($P = 0.09$). However, body weight change on rosiglitazone was associated with an increase in TBW of 1.6 ± 0.6 l ($P < 0.05$), whereas TBW did not significantly change in the placebo group (-0.2 ± 0.7 l, $P = 0.79$). The difference in TBW change was not significantly different between treatments ($P = 0.06$).

The cumulative nutrient balances are shown in Figures 2(a), (b) and (c). The cumulative protein balance became more positive from day 1 to 7 in both groups (Figure 2a). Total protein balance by the end of day 7 was significantly different from zero for rosiglitazone (1.6 ± 0.5 MJ; $P < 0.01$), but not for placebo (1.0 ± 0.6 MJ; $P = 0.10$). Total protein balance was not significantly different between treatments ($P = 0.47$).

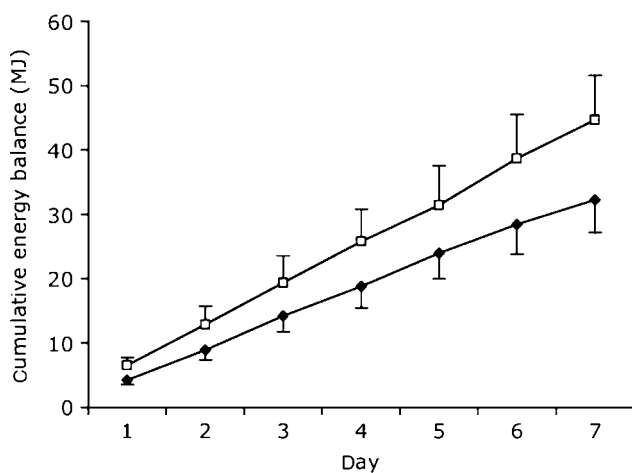


Figure 1. Mean (\pm SE) cumulative energy balance (MJ) for the placebo (\blacklozenge) and rosiglitazone (\square) groups on days 1–7

From day 1 to 4 the cumulative carbohydrate balance increased, but then decreased from day 5 indicating an adaptation of carbohydrate oxidation to carbohydrate intake (Figure 2b). By the end of day 7, total carbohydrate balance was not significantly different from zero for both the placebo (0.8 ± 1.8 MJ, $P = 0.65$) and the rosiglitazone groups (-0.2 ± 1.5 MJ, $P = 0.90$). There was no significant effect of treatment on the carbohydrate balance ($P = 0.67$).

The total EB was for both treatments mainly determined by the fat balance. The cumulative fat balance gradually increased from day 1 to 7 (Figure 2c). By the end of day 7, the cumulative fat balance was 29.5 ± 4.9 MJ ($P < 0.001$) for placebo and 42.3 ± 6.8 MJ ($P = 0.00$) for rosiglitazone. Although this was not significantly different between the treatments ($P = 0.14$), the pattern of fat intake and fat oxidation differed. Figure 3 shows that both groups did not adjust their fat oxidation to the increased fat intake. However, fat oxidation decreased faster in the rosiglitazone group and by day 6 and 7, fat oxidation was significantly lower compared to placebo.

Individual fat balances all increased similarly in the placebo group, independent of the classification normal weight (BMI < 25 kg/m², solid lines) or overweight (BMI ≥ 25 kg/m², dotted lines) (Figure 4a). However, there was a significant interaction between body weight class and treatment ($P < 0.001$). As can be seen in Figure 4(b), during treatment with rosiglitazone overweight subjects stored more fat than normal weight subjects.

Blood parameters

Table 2 shows the mean fasting plasma concentrations of glucose, insulin, leptin and TAGs. In the placebo group, plasma glucose was decreased and TAGs were increased after overfeeding, other parameters did not change significantly. In the rosiglitazone group, plasma insulin and TAGs were increased after overfeeding, plasma glucose and leptin did not change significantly. There was a significant treatment effect on changes in plasma glucose ($P = 0.02$) and TAGs ($P = 0.03$), but not on plasma insulin ($P = 0.07$) and leptin ($P = 0.15$).

Table 2. Fasting plasma parameters at baseline and after self-induced overfeeding for the placebo and rosiglitazone treated groups

	Placebo (n = 10)		Rosiglitazone (n = 10)					
	Baseline		Overfeeding		Baseline		Overfeeding	
	mean	SE	mean	SE	mean	SE	mean	SE
Glucose (mmol/l)	5.0	0.1	4.6 ^a	0.1	4.8	0.1	4.9	0.2
Insulin (μ U/ml)	7.8	0.6	8.5	0.5	6.0	0.8	8.2 ^b	1.1
Leptin (ng/ml)	5.4	1.1	5.6	1.0	5.6	1.4	5.3	1.4
TAG (mmol/l)	0.6	0.04	1.2 ^a	0.2	0.8	0.1	2.5 ^a	0.7

TAG = triacylglycerol

^aSignificantly different from baseline (within-group) using Wilcoxon's paired signed rank test $P < 0.01$.

^b $P = 0.01$.

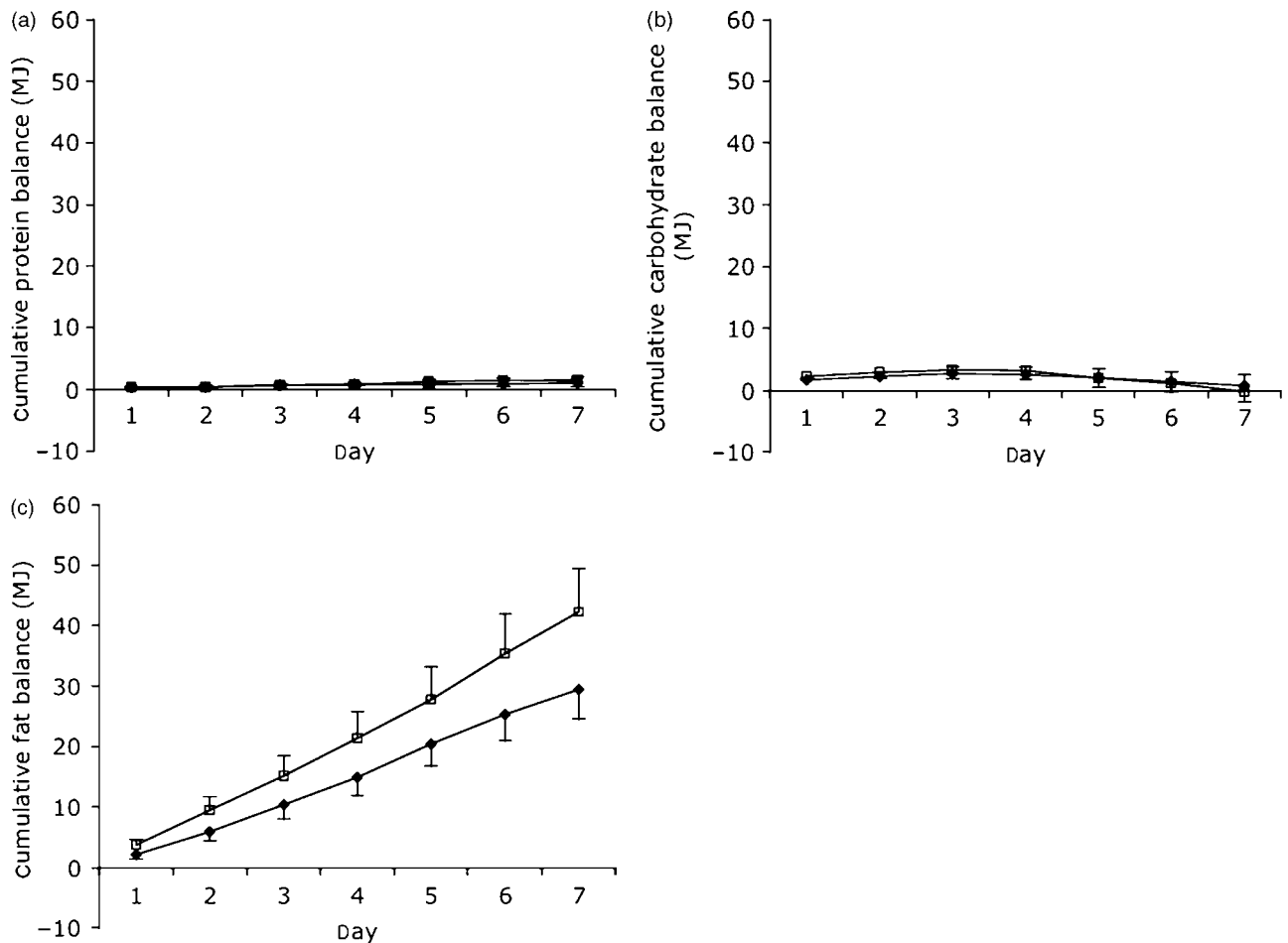


Figure 2. Mean (\pm SE) cumulative protein (a), carbohydrate (b) and fat (c) balances (MJ) for the placebo (◆) and rosiglitazone (□) groups on days 1–7

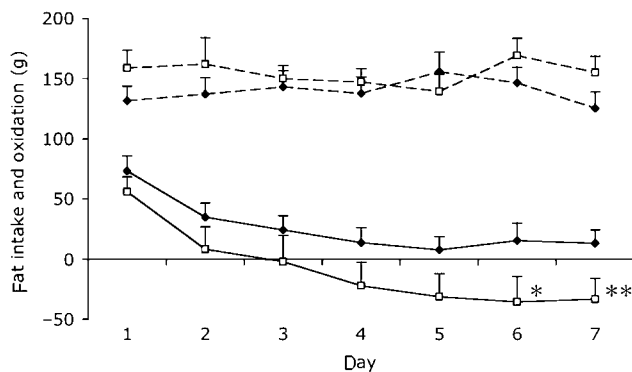


Figure 3. Mean (\pm SE) fat intake (dotted lines) and fat oxidation (solid lines) (g) for placebo (◆) and rosiglitazone (□) groups on days 1–7. * $P = 0.05$, ** $P < 0.05$ oxidation between groups (one-way ANOVA)

Discussion

The increasing prevalence of obesity is related to changing dietary habits and a sedentary lifestyle [22]. The contribution of environmental factors is apparent, but genetic factors are also involved [23]. PPAR γ is a key regulator of fat metabolism; therefore, a high PPAR γ activity could form a predisposing factor for

the excess of fat mass characteristic for obesity. The present study aimed to investigate the influence of the PPAR γ ligand rosiglitazone on substrate usage, EE and EI. Twenty males were studied during a 7-day stay in the respiration chamber. During this period, PPAR γ activity was stimulated with rosiglitazone in ten males; the control group received a placebo treatment.

As shown before [24,25], the sedentary lifestyle imposed by the restrictions of the small environment in the respiration chamber together with an *ad libitum* food intake resulted in a significant positive EB by the end of day 7 in both groups. Though not statistically significant, there was a trend towards an effect of PPAR γ stimulation on EB regulation. EI tended to be higher in the rosiglitazone treated group, similar to results observed in lean rats, in diet-induced obese rats, and in insulin-resistant fatty Zucker rats treated with TZD, where an increased weight gain was associated with increased food intake and feed efficiency [13–15]. Theoretically, PPAR γ can influence food intake through a direct effect on the central nervous system or indirectly through effects on peripheral tissues. In humans, PPAR γ is most abundantly expressed in adipose tissue and at lower levels in skeletal muscle, liver and heart [2,3]. It is therefore most likely that the tendency to increase food intake with

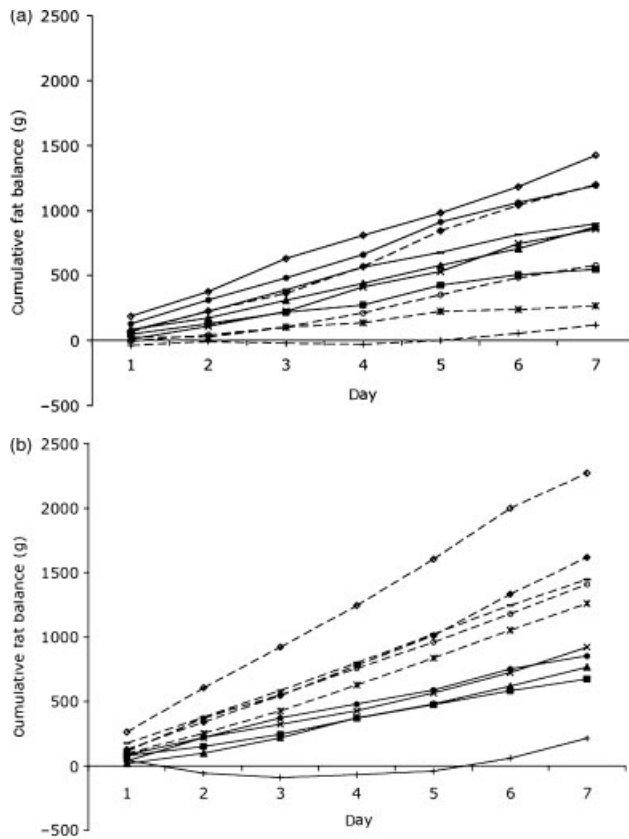


Figure 4. Individual cumulative fat balances (g) for the placebo (a) and rosiglitazone (b) groups. Solid lines represent subjects with BMI < 25 kg/m², dotted lines represent subjects with BMI ≥ 25 kg/m²

rosiglitazone is because of the PPAR γ activity in adipose tissue. The adipokine leptin is known to be a regulator of EI and expenditure [1], and plasma leptin concentrations can be decreased in lean and diet-induced rats with PPAR γ stimulation [13]. However, plasma leptin was not decreased in *ad libitum*-fed rats treated with rosiglitazone and could thus not explain the observed hyperphagia [13]. Similarly, plasma leptin did not change on either treatment and is therefore unlikely to have influenced food intake or thermogenesis in our subjects.

There was no statistical significant effect of PPAR γ stimulation on EE, indicating no thermogenic dissipation of the excess energy. However, when we look at nutrient oxidation, there was an effect on fat oxidation after 6 days of rosiglitazone treatment. Both groups could not adjust their fat oxidation to fat intake during the 7-day treatment period, but treatment with rosiglitazone worsened the adaptation of fat oxidation to fat intake. Independent of treatment, the majority of excess energy was stored as fat, as can be expected from the limited storage capacity for carbohydrates which forces an increase in carbohydrate oxidation to maintain carbohydrate balance. In addition, the positive fat balances seen in the majority of the subjects developed faster in overweight subjects compared to normal weight subjects treated with rosiglitazone. Smith *et al.* [26] found similar results in obese type 2 diabetic patients treated with

another TZD, pioglitazone (45 mg/d), for 24 weeks. Though not statistically significant, substrate oxidation after a meal tended towards fat storage and an increased carbohydrate oxidation. Pioglitazone treatment resulted in fat gain, but this change could not be accounted for, as measurements of 24-h EE and EI were not included. In contrast, Füllert *et al.* [27] did not find an increase in body weight in overweight, non-diabetic patients with arterial hypertension treated with 45 mg/d pioglitazone for 16 weeks.

Furthermore, overfeeding resulted in increased fasting plasma TAG concentrations in both groups, but this increase was significantly higher with PPAR γ stimulation. As fasting plasma TAGs and fat oxidation have been shown to predict weight gain in diet-induced prone and resistant rats [28], this indicates an insufficient fat oxidation and a tendency to retain TAGs in the plasma until storage in the adipose tissue. However, PPAR γ activation during *ad libitum* feeding, initiating a positive EB, resulted in lower plasma TAGs in db/db mice, fatty Zucker rats [10], ob/ob mice [29] and diet-induced mice [14] but not in lean mice [29], which suggests that fat storage upon PPAR γ activation is only accompanied by a decrease in plasma TAGs if baseline values are already elevated.

The favourable effects of TZD treatment on plasma glucose and insulin concentrations in type 2 diabetic patients are attributed to their ability to stimulate PPAR γ [9]. However, in our group of healthy, normal to overweight men, overfeeding induced a significant increase in plasma-insulin concentrations with PPAR γ stimulation. Probably, TZD treatment is only associated with decreased insulin in patients already showing elevated plasma concentrations. This was also seen in patients with type 1 diabetes, where the greatest improvements in glycemic control with combined rosiglitazone and insulin treatment were seen in those patients with more pronounced markers of insulin resistance [30].

Though PPAR γ activity in adipose tissue is necessary to convert an energy surplus into fat mass, PPAR γ does play an important role in liver and muscle fat metabolism as well. Adipose-specific PPAR γ knockout mice are lipodystrophic, leading to decreased plasma leptin and increased plasma TAG concentrations, storage of fat in skeletal muscle and liver, and increased hepatic PPAR γ mRNA [31]. Hepatic PPAR γ plays a critical role in the regulation of TAG content, blood glucose homeostasis and insulin resistance as liver-specific PPAR γ knockout mice have a diabetic phenotype including an increased adiposity [32,33]. PPAR γ knockout in muscle, the major site of fat oxidation, causes an impaired ability of muscle to use fat and thus shunting of fat to the liver, increased adiposity and hepatic insulin resistance. Our results indicate that healthy men treated with rosiglitazone develop an insulin-resistant state, with increased plasma TAG and insulin concentrations, which facilitates the development of a positive fat balance. The observation that overweight subjects develop a positive fat balance faster than do normal weight subjects supports this hypothesis, as overweight subjects are generally

considered to be obesity-prone. We expect that these effects will be more pronounced when rosiglitazone treatment continues for longer periods as it might take more than 7 days for full effects on plasma parameters to develop. In addition, fat oxidation gradually became more suppressed compared to the placebo group from day 6, which might have been earlier if rosiglitazone treatment was already started before the respiration chamber measurements. Yet, TBW was significantly increased in the rosiglitazone group, which indicates that rosiglitazone exerted effects on metabolism within 7 days.

In summary, our results suggest a shift in substrate usage during PPAR γ stimulation leading to a preference for fat storage, especially in subjects with a higher BMI.

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