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Regulation of Adipokine Production in Human Adipose Tissue by Propionic Acid

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Abstract

Background: Dietary fiber (DF) has been shown to be protective for the development of obesity, insulin resistance and type 2 diabetes. Short-chain fatty acids, produced by colonic fermentation of DF might mediate this beneficial effect. Adipose tissue plays a key role in the regulation of energy homeostasis, Therefore, we investigated the influence of the short-chain fatty acid propionic acid (PA) on leptin, adiponectin and resistin production by human omental (OAT) and subcutaneous adipose tissue (SAT). As PA has been shown to be a ligand for G-protein coupled receptor (GPCR) 41 and 43, we investigated the role of GPCR's in PA signaling.

Materials and methods: Human OAT and SAT explants were obtained from gynecological patients who underwent surgery. Explants were incubated for 24 hours with PA. Adipokine secretion and mRNA expression were determined by ELISA and RT-PCR, respectively.

Results: We found that PA significantly stimulated leptin mRNA expression and secretion by OAT and SAT, whereas it had no effect on adiponectin. Furthermore, PA reduced resistin mRNA expression. Leptin induction, but not resistin reduction, was abolished by inhibition of Gi/o-coupled GPCR signaling. Moreover, GPCR41 and GPCR43 mRNA levels were considerably higher in SAT than in OAT.

Conclusions: We demonstrate that PA stimulates expression of the anorexigenic hormone leptin and reduces the pro-inflammatory factor resistin in human adipose tissue depots. This suggests that PA is involved in regulation of human energy metabolism and inflammation and in this way may influence the development of obesity and type 2 diabetes.

Key words: Propionic acid, Human adipose tissue, G-protein coupled receptors, Leptin, Adiponectin, Resistin.

Introduction

Obesity and its associated disorders, such as insulin resistance, type 2 diabetes and cardiovascular diseases, have become major public health issues. The etiology of obesity and insulin resistance is complex and involves life style factors such as physical activity and diet. The dietary fiber content (DF) of the diet is one of the factors that may influence the development of these diseases. Several studies show that consumption of DF prevents accumulation of fat mass [1-3] and increases insulin sensitivity [4-6]. This may be due to fermentation of DF by the colonic-microbiota leading to the production of metabolites such as short chain fatty acids (SCFA). SCFA are rapidly absorbed from the colonic lumen and partly metabolized by colonic epithelial cells. The rest enters the portal and peripheral circulation [7]. The finding that SCFA are ligands for the G protein-coupled receptor GPCR41 and GPCR43, present on adipose tissue [8-9], suggests that adipose tissue is an important target for SCFA. As adipose tissue is an endocrine organ that produces a great variety of adipokines, that influence metabolism in other organs, it could constitute a link between colonic fermentation and peripheral metabolic effects. Only few studies so far investigated the direct effect of SCFA on factors involved in regulation of energy metabolism. It has been demonstrated that propionic acid (PA) stimulates the production of the anorexigenic adipokine leptin in mouse and bovine adipocytes *in vitro* [10-12] and sheep adipose tissue *in vivo* [13]. In addition, dietary supplementation of PA has been shown to reduce food intake in chickens and cows [14-15]. Very recently, dietary supplementation with butyrate, another SCFA, has been shown to prevent weight gain in mice by increasing among others fat oxidation and energy expenditure [16].

Until now, all studies investigating effects of SCFA are performed in animals or animal tissue. Therefore, the aim of our study was to determine the effect of PA on the expression and secretion of adipokines by human adipose tissues. As different adipose tissue depots may vary in adipokine secretion patterns, we used omental as well as subcutaneous tissue. In addition to leptin, which is known to reduce food intake and increase energy expenditure [17] we examined the effect on adiponectin and resistin. Adiponectin has insulin sensitizing and anti-inflammatory effects [18]. Resistin is a pro-inflammatory factor [19-20] and recently, it has been associated with insulin

sensitivity [21-22]. Moreover, we studied the involvement of the GPCR signaling pathway in the regulation of adipokine response to PA.

Materials and methods

Materials

Gentamycin, glucose, PA and pertusis toxin (PTX) were purchased from Sigma (Zwijndrecht, The Netherlands). M199 media was purchased from Invitrogen (Breda, The Netherlands). GPCR43 and GPCR41 primers were purchased from Applied Biosystems (Nieuwerkerk a/d IJssel, The Netherlands); whereas the other primers were purchased from Biolegio (Nijmegen, The Netherlands).

Human adipose tissue culture

Human omental and abdominal subcutaneous adipose tissue explants as well as serum samples were obtained from 28 females, who underwent surgery for gynecologic disorders such as myoma, endometriosis and refertilization. None of the women had diabetes and their characteristics are summarized in Table 1. The study was approved by the local medical ethical committee.

Adipose tissue culture was performed as described previously [23] with slight modifications. 0.5 g tissue/5 ml of M199 medium was used and after the last washing step, tissue explants were incubated for 24 hours with different PA concentrations (0, 0.01, 0.1, 1, 3 and 10 mM). Regarding PTX treatment, adipose tissue explants were pre-incubated with PTX (100 ng/ml) for 2 hours. Thereafter, 3 mM PA was added and incubated for 24 hours. Subsequently, tissue was frozen immediately in liquid nitrogen and then stored at -80°C until RNA was isolated. Media samples were stored at -80°C prior to ELISA measurements.

Relative Q-PCR analysis

Total RNA was isolated from adipose tissue by the RNeasy lipid tissue mini kit and cDNA was synthesized using the Quantitect kit (Qiagen, Venlo, Netherlands). Relative quantification of genes were performed in triplicate with the ABI 7900HT sequence detection system for relative real time polymerase chain reaction (Taqman, Applied Biosystems) using the ΔΔCT method. The primers pairs and probes used are displayed in Table 2, except for GPCR41 and 43, which were obtained from Applied Biosystems (ID numbers Hs00271131_s1 and Hs00271142_s1, respectively). Stability of several housekeeping genes was assessed by geNorm analysis software (http://medgen.ugent.be/~jvdesomp/genorm/) [24]. GAPDH was chosen as the most stable

housekeeping gene expressed in adipose tissue. PCR was performed using TaqMan Universal Master Mix in a total reaction mix volume of 10 µL. The PCR conditions were: 15 minutes at 95 °C, 40 cycles of 15 seconds at 95 °C followed by 1 minute at 62 °C.

Adipokine protein quantification

Leptin and adiponectin concentrations were measured in culture media by Duoset ELISA kit in duplicate, according to the manufacturer's description (R&D Systems, Abingdon, United Kingdom); while resistin was measured in culture media and serum by Quantikine ELISA kit (R &D Systems).

Statistics

All data are given as mean ± SEM. Comparison between two groups was performed by two-sided paired Student's t-test. The correlation between adipokine mRNA expression and protein level, age and anthropometric indices (body mass index (BMI), waist circumference (WC), hip circumference (HC) and waist to hip ratio (WHR), was calculated using the Pearson's product moment correlation coefficient test. Results were considered to be statistically significant when $P < 0.05$.

Results

PA dose-effect relationship with adipokine mRNA expression in omental adipose tissue

OAT explants derived from 13 subjects were used to determine the dose-effect relationship of PA on adipokine mRNA expression after incubation for 24 hrs. Figure 1 shows that leptin mRNA expression was significantly stimulated 65% and 100% by 1 mM (P = 0.04) and 3 mM (P = 0.006) PA treatments, respectively. In contrast, 10 mM did not influence leptin mRNA expression. This was also noticed by Le Poul et al [9], who found that 10 mM did not influence neutrophils chemotaxis, while 1 mM did. On the other hand, resistin mRNA expression was significantly down-regulated 44% (P = 0.0026) and 94% ($P < 0.0001$) in adipose tissue treated with 3 and 10 mM PA, respectively. None of the PA concentrations affected adiponectin mRNA expression.

PA effect on adipokine mRNA expression and protein secretion in omental adipose tissue

Based on the results above, 3 mM PA was chosen for additional experiments in an extended set of explants to investigate the influence of age and anthropometric indices of the explant donors on the magnitude of the PA effect on adipokine mRNA and protein expression. As represented in Figure 2, leptin mRNA (N=27) and protein (N=12) levels were significantly induced by ~90% ($P < 0.0001$ for both); whereas resistin expression was significantly reduced (N=27; $P < 0.0001$) on the mRNA level by 46%, but not on the protein level (N=12; P = 0.61). With respect to adiponectin, neither mRNA (N=22) nor protein levels (N=12) were changed. Induction of leptin mRNA expression and protein secretion after PA treatment of OAT was independent of age or anthropometric indices of the donors (Table 3). Basal resistin mRNA expression in OAT was positively correlated to the donors' age (R = 0.49, P = 0.013) but not to anthropometric indices, whereas the response of resistin to PA was negative correlated to the donors' age only (R = -0.41, P = 0.03). While there was a positive correlation between basal resistin mRNA expression in adipose tissue with age, no correlation was observed between serum resistin and age of the explant donors.

PA effect on adipokine mRNA expression and protein secretion in subcutaneous adipose tissue

The effect of PA was also investigated on adipokine mRNA and protein levels in SAT derived from 5 and 12 subjects, respectively. As depicted in Figure 2, leptin protein and mRNA levels were induced by approximately 35% (P = 0.012 and 0.027, respectively), resistin was reduced only on the mRNA level by 32% (P = 0.003) and adiponectin was unaffected both on the mRNA and protein level.

GPCR41 and GPCR43 expression in human omental and subcutaneous adipose tissues

SCFA are ligands for the G protein-coupled receptor GPCR41 and GPCR43 [8-9]. Therefore, we determined their expression in human OAT and SAT. As shown in Figure 3, we found that GPCR41 was expressed at similar levels as GPCR43 in each adipose depot. However, levels of both receptors in SAT were approximately 10-fold higher than in OAT (P = 0.009 and 0.021 for GPCR41 and GPCR43, respectively). We also determined whether GPCR41 and GPCR43 expression in OAT was correlated to age and anthropometric indices of the donors (Table 3). GPCR41 mRNA expression had a significant and positive correlation with WHR (R = 0.48, P = 0.03); whereas GPCR43 expression correlated negatively to HC (R = -0.53, P = 0.013).

Involvement of Gi/o-protein coupled receptors in the effect of PA on leptin and resistin mRNA expression

In mouse adipocytes, it has been shown that PA mediates its effect on leptin production via GPCR41, a Gi/o-protein coupled receptor (Gi/o-PCR) [10]. Gi/o-proteins belong to one of four classes of G-proteins that are coupled to their corresponding receptors and mediate their intracellular signals. To determine whether PA uses GPCR coupled to Gi/o proteins to affect leptin and resistin expression in human adipose tissue, OAT and SAT from six and five subjects, respectively, were pretreated with PTX to block the Gi/o pathway. Figure 4 illustrates that induction of leptin expression by PA was completely abolished by PTX pretreatment in both OAT and SAT, while the PA-induced reduction in resistin expression was not affected by PTX. PTX treatment also reduced baseline leptin expression by approximately 60% and 40% in both OAT and SAT, respectively (P = 0.004 and 0.01,

respectively). In OAT, PA plus PTX could not completely abolish leptin mRNA expression to the level observed with PTX alone.

Discussion

The present human *ex vivo* study reveals that PA influences adipokine production by both adipose tissue depots (OAT and SAT). Explants were derived from women without diabetes with BMI's ranging between 20.8 and 33.3 Kg/m^2. PA induced leptin, both on the mRNA and protein level, while it almost completely abolished resistin mRNA expression. On the other hand, adiponectin expression was not influenced. The magnitude of the PA effect on leptin was 1.4 fold higher in OAT compared to SAT and was independent of the degree of adiposity and age of the explant donors. Induction of leptin by PA may indicate that PA reduces food intake and increases energy expenditure, since leptin is a potent anorexigenic hormone [17]. In sheep, PA has been shown to induce leptin expression in adipose tissue [25] and to reduce food intake [26]. The observed PA-induced reduction of resistin mRNA expression was slightly more pronounced in OAT compared to SAT and was negatively associated with age but independent of the degree of adiposity of the explant donors. However, on the protein level no significant change in resistin expression was seen. This discrepancy between the mRNA and the protein level maybe explained by the fact that resistin is only released from the cell surface membrane or intracellular stores after an inflammatory trigger [27]. Therefore, a reduction in resistin protein only can be observed when cellular stores are depleted. Resistin is pro-inflammatory and, in human adipose tissue, is predominantly produced by macrophages [28]. Resistin is also a marker for insulin resistance in humans [21, 22]. The PA-induced decrease in resistin expression may imply that PA is acting as an anti-inflammatory agent. This is in agreement with other studies in which PA has been shown to have anti-inflammatory effects on human neutrophils and mouse colonic organ cultures [29]. Furthermore, PA inhibits proliferation of granulocyte/macrophage, hematopoietic progenitor cells and lymphocyte activation [30-32]. The observed improvement of insulin sensitivity in humans after high DF diets [4-6] may be attributed to this anti-inflammatory effect of PA since in obese persons adipose tissue inflammation and insulin resistance are closely linked [33].

The SCFA receptors GPCR41 and GPCR43 are implicated in mediating the PA effect. Our results show that both human adipose tissue depots expressed GPCR41 and GPCR43 mRNA. SAT

expressed approximately 10-fold higher amounts of each receptor than OAT, suggesting that SAT is more repsonsive to PA than OAT. However, we found slightly higher responses to PA in OAT compared to SAT. Brown et al [8] and Le Poul et al [9] also showed GPCR41 expression in human adipose tissue. However the adipose tissue depot was not specified. To our knowledge, there are no studies that have reported expression of GPCR43 in human adipose tissue before. GPCR43 was however detected in immune cells [8, 9] and in mouse adipose tissue and adipocytes [11]. Which cell types are responsible for the observed GPCR43 expression in human adipose tissue remains to be determent. Furthermore, we found that GPCR41 mRNA level in OAT was positively associated with WHR, as a measure of adiposity, while GPCR43 was negatively associated with HC (Table 3). However, no correlation with other measures of obesity (BMI and WC) was found. Therefore, the relevance of this observation remains unclear.

PTX abolished leptin induction by PA in both human OAT and SAT similar to what has been observed in mouse adipocytes [10]. This suggests that PA regulates leptin production through GPCR coupled to the Gi/o signaling pathway. However, the effect of PA on resistin expression was not inhibited by PTX. Le Poul et al. [9] showed that there is a unique Gi/o coupling for GPCR41 but for GPCR43, a dual coupling exists through Gi/o and the PTX insensitive Gq. Furthermore, in human adipose tissue, resistin is almost exclusively expressed in macrophages [28] while leptin expression is specific for adipocytes [34]. This may suggest that leptin induction by PA is mediated via GPCR41 on adipocytes, coupled to Gi/o, while the reduction of resistin by PA is mediated via GPCR43 on resident macrophages, coupled to Gq.

In *in vitro* studies [8-11], including our study, relatively high PA concentrations (1-10 mM) are needed for the observed effects. The question rises whether colonic fermentation can provide such concentrations. Due to the absence of blood flow the effective concentration in tissue cultures probably is substantially higher than *in vivo.* The PA concentration in the human colon is approximately 20 mmol/kg [7]. In ruminants, 50% of absorbed PA reaches the portal vein [35], indicating that the remainder is utilized by tissues it encounters such as the colonic wall and possibly OAT. I*n vivo,* the exposure time of adipose tissue to PA may be an important factor. Furthermore,

effects of different SCFA may be additive as acetate, propionate and butyrate all share the same receptors [8, 9]. Further *in vivo* measurements have to confirm this.

In conclusion, the present study demonstrates for the first time that PA influences adipokine secretion by human adipose tissue. We show that leptin and resistin, but not adiponectin production is affected by PA and involves GPCR signaling. These findings suggest that modulation of PA quantity through e.g. dietary manipulation, prebiotics and probiotics, or dietary supplementation may have potential in preventing obesity and its associated complications such as inflammation and insulin resistance.

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Table 1 Characteristics of adipose tissue donors (N=28).

Characteristics	Mean	Range
Age (years)	45.8 ± 9.3	26-68
body mass index (BMI, Kg/m^2)	26.3 ± 3.3	20.8-33.3
Waist circumference (WC, cm)	87.3 ± 11.4	57-104
Hip circumference (HC, cm)	102.4 ± 9.7	82-120
Waist to hip ratio (WHR)	0.9 ± 0.08	0.54-0.93

Table 2 Primer sequences

Primer ID	Primer sequence (5'➔3')
Leptin forward	TCA CCA GGA TCA ATG ACA TTT CAC
Leptin reverse	AGC CCA GGA ATG AAG TCC AAA C
Leptin probe	CGC AGT CAG TCT CCT CCA AAC AGA AAG TCA
Adiponectin forward	AGG CCG TGA TGG CAG AGA T
Adiponectin reverse	GTC TCC CTT AGG ACC AAT AAG ACC T
Adiponectin probe	ATC TCC TTT CTC ACC CTT CTC ACC AGG G
Resistin forward	AAG CCA TCA ATG AGA GGA TCC A
Resistin reverse	CTC CAG GCC AAT GCT GCT TA
Resistin probe	CCC TAA ATA TTA GGG AGC CGG CGA CCT C
GAPDH forward	GGT GAA GGT CGG AGT CAA CG
GAPDH reverse	ACC ATG TAG TTG AGG TCA ATG AAG G
GAPDH probe	CGC CTG GTC ACC AGG GCT GC

Table 3 Analysis of the correlations (r) in OAT between the mRNA expression levels of GPCR41, GPCR43 and resistin, resistin level in serum and resistin and leptin responses to 3 mM PA on the one hand and age and anthropometric indices on the other hand.

	GPCR41 basal mRNA levels	**GPCR43 basal mRNA levels**	**Resistin basal mRNA levels**	**Resistin basal serum levels**	**Resistin mRNA response to PA**	**Leptin mRNA response to PA**
BMI	0.078	-0.23	0.088	0.29	-0.3	0.037
WC	0.099	-0.37	0.076	0.17	-0.14	0.11
HC	-0.15	-0.53*	0.22	0.17	-0.17	-0.17
WHR	0.48*	0.2	-0.083	0.035	-0.025	0.32
Age	-0.023	0.05	0.49*	0.25	-0.41*	-0.015

BMI: Body mass index, HC: hip circumference, WC: waist circumference and WHR: waist-hip ratio.

* $P < 0.05$.

Figure 1 Dose-response curves of PA on adipokine production by human OAT. Human OAT explants were incubated in culture for 24 h with the indicated concentrations of PA in triplicate. Leptin (■), adiponectin (●) and resistin (▲) mRNA expression levels were determined by RT-PCR and depicted as relative quantities compared to controls (without PA). Error bars represent SEM.* $P < 0.05$.

Figure 2 PA effects on adipokine production by OAT and SAT. 3 mM PA (■) was chosen to validate the effect of PA on adipokine production by OAT on both mRNA (a) and protein (b) levels. Human adipose tissue in culture was incubated for 24 h with 3 mM PA in triplicate. mRNA expression levels were determined by RT-PCR and secreted adipokine levels in the media were determined by ELISA. Results are depicted as relative quantities compared to controls (without PA; □). Error bars represent SEM. * $P < 0.001$; + $P < 0.0001$ and ‡ $P < 0.05$.

Figure 3 Gene expression levels of GPCR41 and GPCR43 in OAT and SAT. mRNA expression of each receptor in SAT was determined by RT-PCR and depicted as a relative quantity compared to OAT. Error bars represent SEM. * $P < 0.05$.

Figure 4 Involvement of G-protein coupled receptor(s). The involvement of G-protein coupled receptors in mediating PA effects on adipokines expression was determined by blocking the Gi/o signaling pathway with PTX. OAT and SAT explants (N=6 and 5, respectively) were incubated in triplicate with PTX (100 ng/ml) for 2 hours before incubation for 24 hours with or without 3 mM PA. mRNA expression levels were determined by RT-PCR and depicted as relative quantities compared to controls (without PA). Error bars represent SEM. * $P \leq 0.001$ vs control; ‡ $P < 0.01$ vs control; § $P < 0.01$ vs PA + PTX, # $P < 0.05$ vs PTX and + $P < 0.05$ vs control.

Figure 1

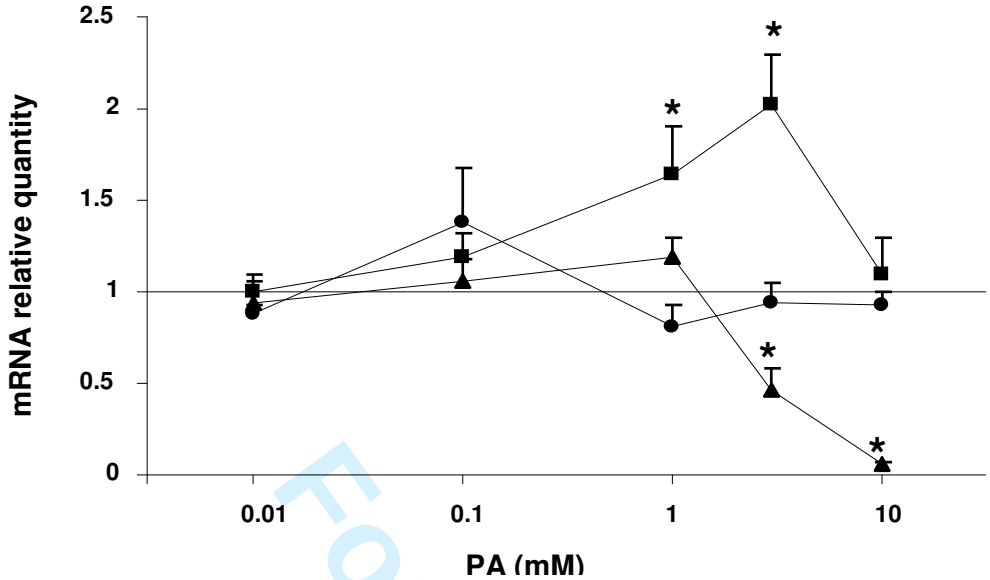

Figure 2

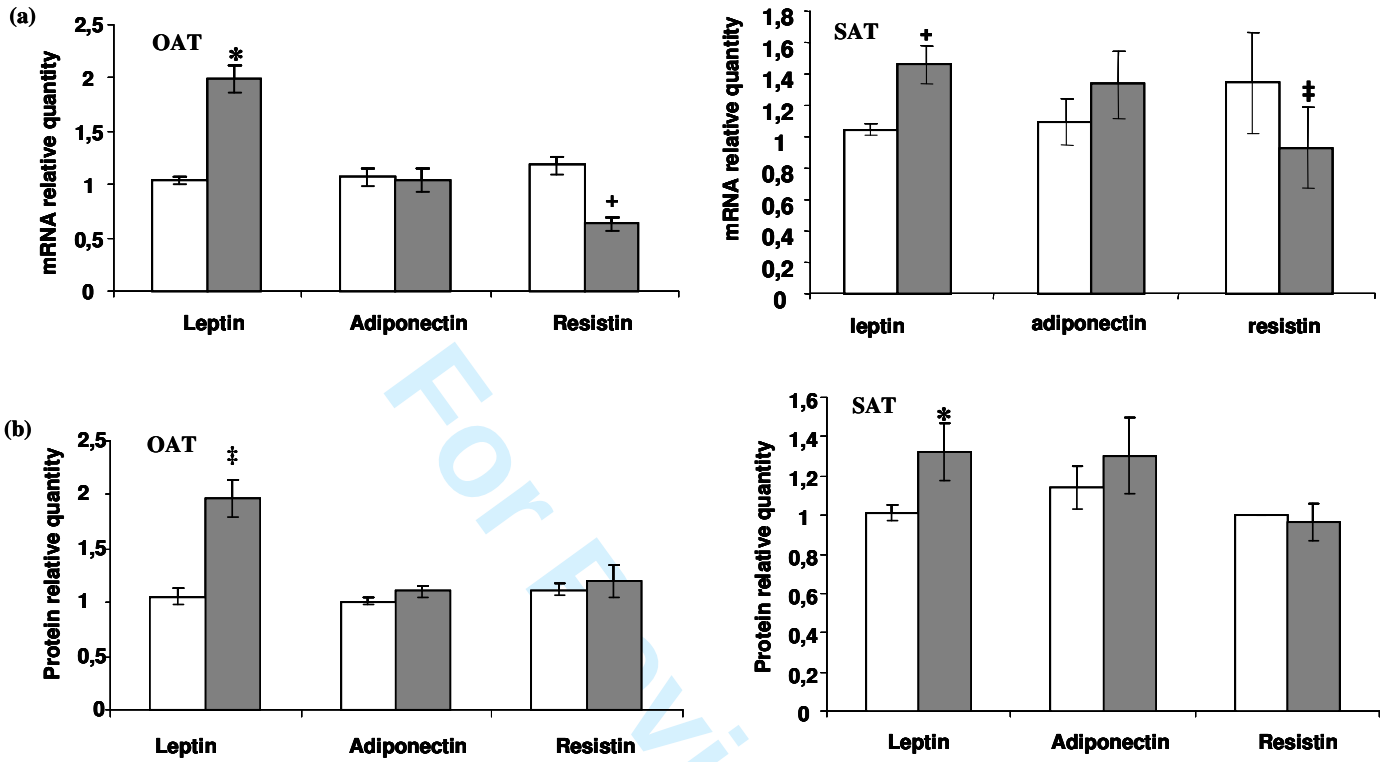

Figure 3

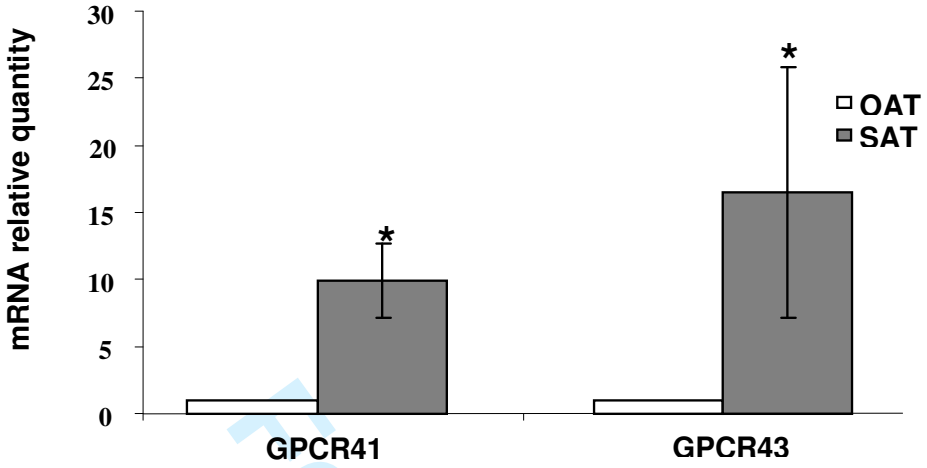

Figure 4