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Subtherapeutic Dose of Pioglitazone Reduces Expression of Inflammatory Adipokines in db/db Mice

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Key Words
Adipocytokines, inflammatory · Adiponectin · Insulin resistance · Pioglitazone · Resistin

Abstract
Agonists of the thiazolidinedione class of peroxisome proliferator-activated receptor-γ exhibit both insulin-sensitizing and anti-inflammatory effects. We hypothesized that pioglitazone might be able to exert its anti-inflammatory properties at a lower dose than that required for its insulin-sensitizing effect. In order to investigate this hypothesis, we evaluated the effects of pioglitazone on inflammatory as well as metabolic biomarkers in serum and white adipose tissue (WAT) at 2 different doses. Female db/db mice were treated orally with therapeutic (30 mg/kg) and subtherapeutic (3 mg/kg) doses of pioglitazone for 14 days followed by an oral glucose tolerance test. Other parameters measured were inflammatory markers such as tumor necrosis factor (TNF)-α, interleukin-6 (IL-6) and metabolic biomarkers in serum (insulin, glucose and adiponectin). Moreover, adiponectin, fatty acid-binding protein (aP2) and lipoprotein lipase (LPL) mRNA expression in WAT were determined by real-time PCR. A subtherapeutic dose of pioglitazone significantly suppresses the expression of TNF-α and IL-6 mRNA in WAT, but does not alter the serum glucose, insulin and WAT expression of adiponectin, adipocyte aP2 and LPL. A therapeutic dose of pioglitazone improves insulin sensitivity, enhances LPL, aP2 and adiponectin expression, and also suppresses TNF-α and IL-6 expression. In conclusion, the current study indicates that the anti-inflammatory effect of pioglitazone is produced at a subtherapeutic dose, which is considerably lower than the dose needed to produce any desired metabolic effects. Anti-inflammatory effects of pioglitazone may precede its insulin-sensitizing effects in db/db mice.

Introduction

Increased adiposity is associated with chronic low-grade inflammation as indicated by an increased expression of inflammatory markers, particularly in adipose tissues [1, 2]. White adipose tissue (WAT) is no longer considered as an inert tissue mainly devoted to energy storage, but has emerged as an active participant in regulating physiological and pathological processes, including immunity and inflammation [2, 3]. Adipose tissue produces and releases a variety of proteins termed adipokines which include leptin, resistin, tumor necrosis factor (TNF)-α, interleukin (IL)-6 and monocyte chemoattractant protein-1 [4, 5]. TNF-α and IL-6 produced by adi-

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pose tissue have been implicated as active participants in the development of insulin resistance associated with obesity and impaired insulin transduction [6–10]. Other adipokines such as adiponectin, leptin, resistin and visfatin have been postulated to play a role in the pathogenesis of insulin resistance [11] and are also altered in a variety of inflammatory conditions [12–15].

Thiazolidinedione (TZD) analogs, which act as peroxisome proliferator-activated receptor (PPAR)-γ agonists, have been established as insulin-sensitizing drugs used in therapy of type 2 diabetes. PPAR-γ is highly expressed in WAT with lower levels in skeletal muscle and liver [16]. PPAR-γ activators are key promoters of adipogenesis [17, 18], improve insulin sensitivity and dyslipidemia, and increase body weight [19–21]. TZDs regulate various genes involved in adipocyte differentiation or lipid and glucose homeostasis. TZDs are also known to modulate various adipokines [22–24]. In addition to these metabolic effects, PPAR-γ agonists are also reported to have anti-inflammatory properties [25–29]. It was reported that rosiglitazone showed anti-inflammatory effects in human type 2 diabetes patients at a 2-mg dose, which is lower than the dose routinely administered to produce antihyperglycemic and insulin-sensitizing effects [26]. This indicated 2 distinct roles of TZDs.

The purpose of the present study is to evaluate whether the anti-inflammatory and insulin-sensitizing effects of pioglitazone occurred at 2 distinctly different dose levels, i.e. subtherapeutic (3 mg/kg) and therapeutic (30 mg/kg). The current study used db/db mice, a model of type 2 diabetes associated with low-grade inflammation.

Materials and Methods

Animals and Treatments

All animal experiments were carried out in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, using protocols approved by the Institutional Animal Ethics Committee (IAEC).

This study was performed in female db/db mice aged 8–10 weeks. The animal colonies were originally procured from the Jackson Laboratory, Bar Harbor, Me., USA. The mice were housed in individually ventilated cages and given pelleted food (standard rodent diet; National Institute of Nutrition, Hyderabad, India) and water ad libitum in a temperature (25 ± 3°C)- and humidity (50–70%)-controlled environment with a 12-hour/12-hour dark-light cycle. The animals were randomized into different groups (n = 12) based on glucose levels followed by body weight. The animals were treated orally with a daily pioglitazone dose of 3 and 30 mg/kg or vehicle (0.5% w/v methylcellulose) for 14 days. On day 15, serum and visceral WAT were collected from 6 animals of each group. Fat samples were flash-frozen for real-time PCR and cytokine level estimation. The remaining 6 animals of each group were subjected to an oral glucose tolerance test (OGTT).

Oral Glucose Tolerance Test

The OGTT was performed by oral administration of glucose solution (3 g/kg body weight) in 18-hour-fasted animals after 14 days' pioglitazone treatment. Blood samples were drawn through retro-orbital sinus before (at 0 min) and 30, 60 and 120 min after glucose administration, and analyzed for glucose levels.

RNA Analysis and Quantitative Real-Time PCR

WAT samples were homogenized in Trizol reagent (Invitrogen, Carlsbad, Calif., USA) using a Mixer 301 (Retsch, Haan, Germany). Total RNA was extracted following the manufacturer's protocol. One microgram total RNA from each sample was taken for first-strand cDNA synthesis using a High Capacity cDNA archive kit (Applied Biosystems, Foster City, Calif., USA; part No. 4322171). cDNA from each sample was taken for quantitative real-time PCR using an ABI Prism 7300. 6-Carboxyfluorescein-labeled Taqman probes, namely adiponectin (Mm00456425_m1), resistin (Mm00445641_m1), TNF-α (Mm00443258_m1), IL-6 (Mm00446190_m1), lipoprotein lipase (LPL) (Mm01345523_m1) and fatty acid-binding protein (aP2) (Mm00445880_m1), were used for PCR in the presence of Taqman Universal Mastermix (Applied Biosystems). A VIC-labeled, housekeeping gene, mouse β-actin (part No. 4352341E) probe was procured from Applied Biosystems and coamplified in each sample with every target gene for normalizing the results.

Biochemical Measurements

Serum glucose levels were determined by the GOD/POD method using a biochemical kit (Ranbaxy Laboratories Ltd, Gurgaon, India). Adiponectin (B-Bridge, Mountain View, Calif., USA), resistin (R&D Systems, Minneapolis, Minn., USA) and insulin (Linco Research, Inc., St. Charles, Mo., USA) levels in the serum were measured by ELISA as specified by the manufacturers. For cytokine assay, visceral WATs were homogenized using ice-cold Tris buffer containing 1 mmol/l phenylmethylsulfonylfluoride. Circulating and adipose tissue TNF-α and IL-6 levels were measured by ELISA using kits from BD Biosciences (San Jose, Calif., USA). The tissue TNF-α and IL-6 levels were expressed per milligram of tissue protein, measured using the biuret method (Pointe Scientific, Canton, Mich., USA).

Data and Statistical Analysis

All the data are expressed as mean ± SEM. The statistical analysis was performed using one-way ANOVA followed by the Tukey test used to identify differences between all groups. The area under the curve (AUC) was calculated using Graphpad Prism software (4.0).

Results

Effect of Pioglitazone Treatment on Fasting Serum Glucose, Oral Glucose Tolerance and Insulin

Pioglitazone (30 mg/kg) treatment for 2 weeks showed a significant ($p < 0.05$) reduction in glucose level in overnight-fasted (7.88 ± 0.31 mmol/l; n = 6) versus control

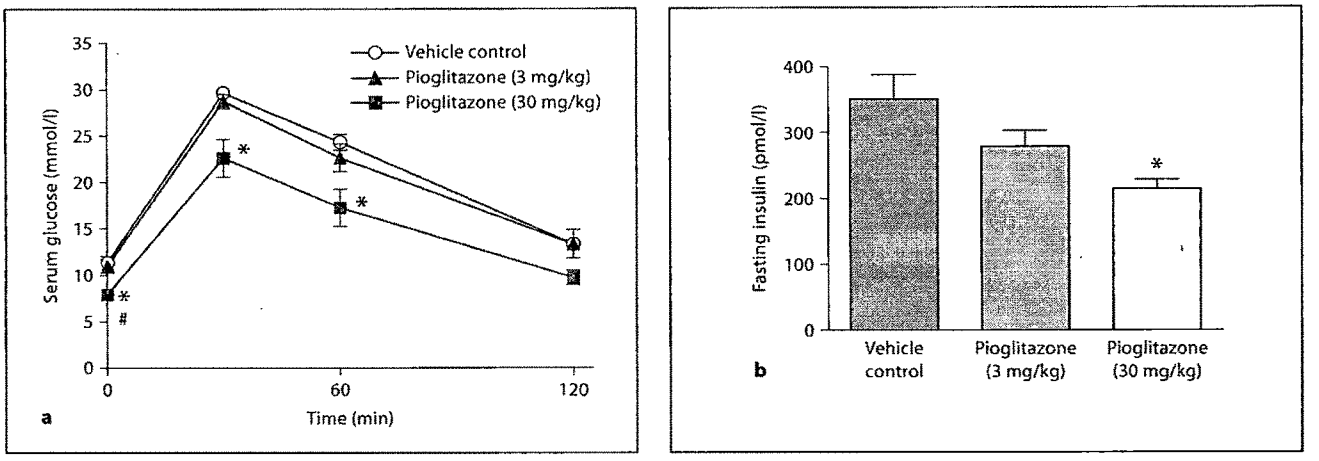


Fig. 1. a Insulin sensitivity was measured using the OGTT in db/db mice. Effect of pioglitazone treatment on serial glucose increase after glucose load in db/db mice. **b** Fasting serum insulin of db/db mice was analyzed by ELISA. Values are means ± SEM (n = 6 mice/group). * $p < 0.05$ compared to vehicle-treated control db/db mice; # $p < 0.05$ for fasting glucose between 3 and 30 mg/kg pioglitazone treatment.

(11.32 ± 0.70 mmol/l; n = 6) db/db mice. However, at a 3-mg/kg dose, there was no significant change in fasting glucose (10.96 ± 0.74 vs. 11.32 ± 0.70 mmol/l; n = 6) compared to the control animals (fig. 1a). There was a statistically significant difference between the pioglitazone groups in fasting glucose after 14 days of treatment.

The AUC calculation based on the OGTT showed a significant ($p < 0.05$) reduction in glucose at the 30-mg/kg dose of pioglitazone when compared to control animals (1,875 ± 123.5 vs. 2,541 ± 210.3; n = 6). On the contrary, 3-mg/kg treatment showed no significant effect (2,438 ± 198.3 vs. 2,541 ± 210.3; n = 6).

Pioglitazone treatment for 2 weeks at 30 mg/kg significantly ($p < 0.05$) reduced the fasting insulin level compared to vehicle control (214.5 ± 13.1 vs. 350.43 ± 34.2 pmol/l; n = 6) at the beginning of the OGTT. However, treatment at 3 mg/kg did not show any significant alteration in insulin levels compared to control (279.1 ± 13.1 vs. 350.43 ± 34.2 pmol/l; n = 6) (fig. 1b).

In the db/db mice treated with pioglitazone (30 mg/kg), 30 min after glucose load, there was a significant ($p < 0.05$) reduction in insulin levels (558.6 ± 29.21 vs. 692.6 ± 37.61 pmol/l; n = 6); however, no significant change was observed at the 3-mg/kg dose when compared to vehicle control (655.7 ± 43.62 vs. 692.6 ± 37.61 pmol/l; n = 6).

Table 1. Changes in body weight in the different groups of db/db mice treated with pioglitazone for 14 days

Groups	Initial body weight	Body weight on day 14	Change in body weight
Vehicle control	39.4 ± 1.1	42.3 ± 0.9	2.9 ± 0.9
Pioglitazone, 3 mg/kg	40.2 ± 0.9	43.6 ± 1.9	3.4 ± 1.4
Pioglitazone, 30 mg/kg	38 ± 1.5	45.4 ± 1.7	7.4 ± 1.3*

Values denote grams and are described as mean ± SEM. * $p < 0.05$ compared to vehicle control.

Effect of Pioglitazone Treatment on Body Weight Gain in db/db Mice

Treatment with the therapeutic dose (30 mg/kg) of pioglitazone caused significant ($p < 0.05$) body weight gain as compared to control animals (table 1). However, no significant effect was found at the 3-mg/kg dose.

Effect of Pioglitazone Treatment on WAT mRNA Expression or Serum Levels of Metabolic Biomarkers (aP2, LPL and Adiponectin)

At the 30-mg/kg dose of pioglitazone, aP2, LPL and adiponectin WAT mRNA expressions were significantly increased ($p < 0.01$) when compared to vehicle-treated animals (fig. 2–4a). At this dose a significant ($p < 0.01$)

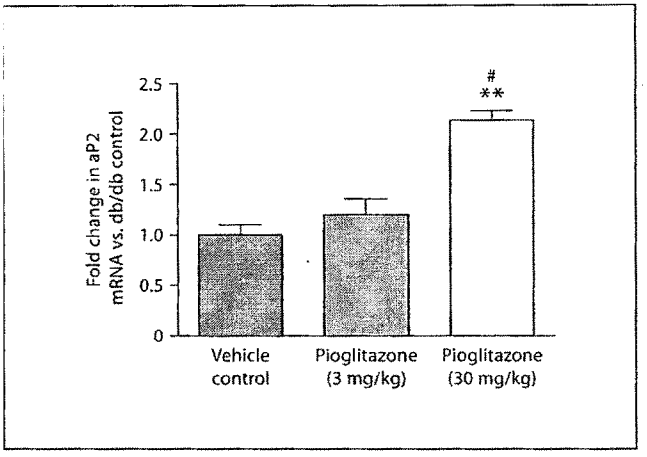


Fig. 2. Expression of aP2 mRNA in WAT of db/db mice was analyzed by quantitative real-time PCR. Fold change in treatment groups vs. vehicle control db/db. Values are means ± SEM (n = 6 mice/group). ** $p < 0.01$ compared to vehicle-treated control db/db mice; # $p < 0.01$ compared to 3 mg/kg pioglitazone.

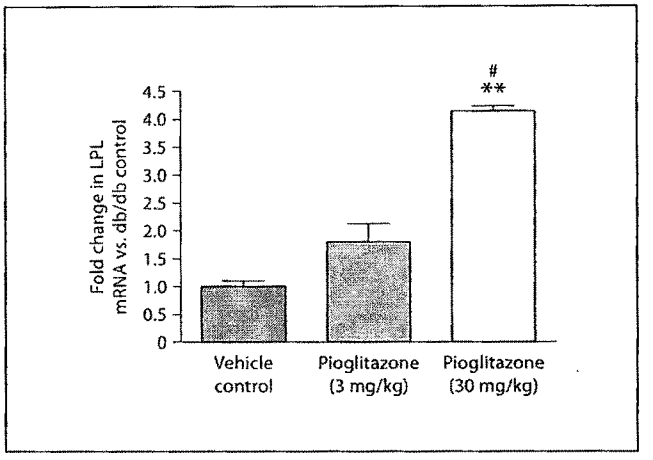
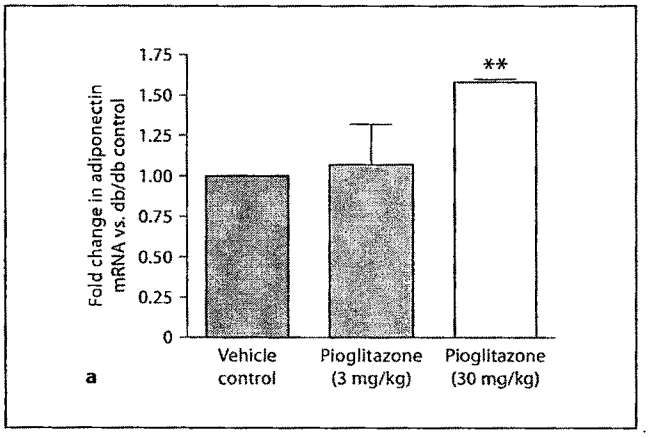


Fig. 3. Expression of LPL mRNA in WAT of db/db mice was analyzed by quantitative real-time PCR. Fold change in treatment groups vs. vehicle control db/db. Values are means ± SEM (n = 6 mice/group). ** $p < 0.01$ compared to vehicle-treated control db/db mice; # $p < 0.01$ compared to 3 mg/kg pioglitazone.



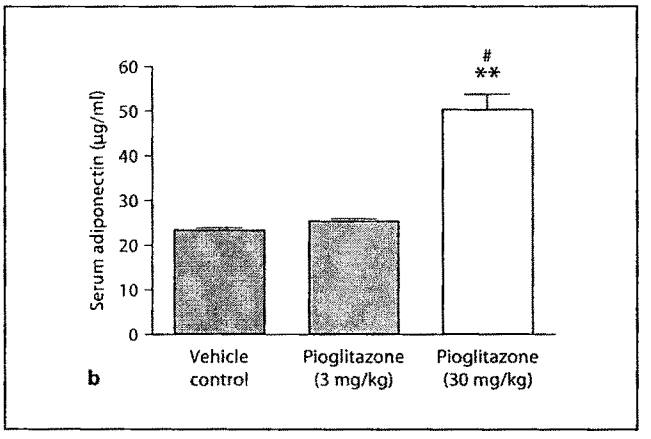


Fig. 4. a Expression of adiponectin mRNA in WAT of db/db mice was analyzed by quantitative real-time PCR. **b** Serum adiponectin in db/db mice was analyzed by ELISA. Values are means ± SEM (n = 6 mice/group). ** $p < 0.01$ compared to vehicle-treated control db/db mice; # $p < 0.01$ compared to 3 mg/kg pioglitazone.

increase in serum adiponectin levels (48.6 ± 3.07 vs. 23.2 ± 0.66 µg/ml; n = 6) was observed which correlated well with the mRNA expression (fig. 4b). No significant changes in mRNA expression or serum levels were observed in the above-mentioned variables at the 3-mg/kg dose. LPL, aP2 mRNA expression and serum adiponectin in the 2 dose groups were significantly different.

Effect of Pioglitazone Treatment on WAT mRNA Expression and Protein Levels of Inflammatory Biomarkers (TNF-α, IL-6 and Resistin)

The 2-week treatment with 3 mg/kg pioglitazone significantly ($p < 0.05$) decreased transcripts of TNF-α and IL-6 in WAT when compared to control animals (fig. 5a, 6a). Serum TNF-α and IL-6 levels were below the detectable limits of the assays used. When adipose tissue levels of TNF-α and IL-6 were measured, the 3-mg/kg piogli-

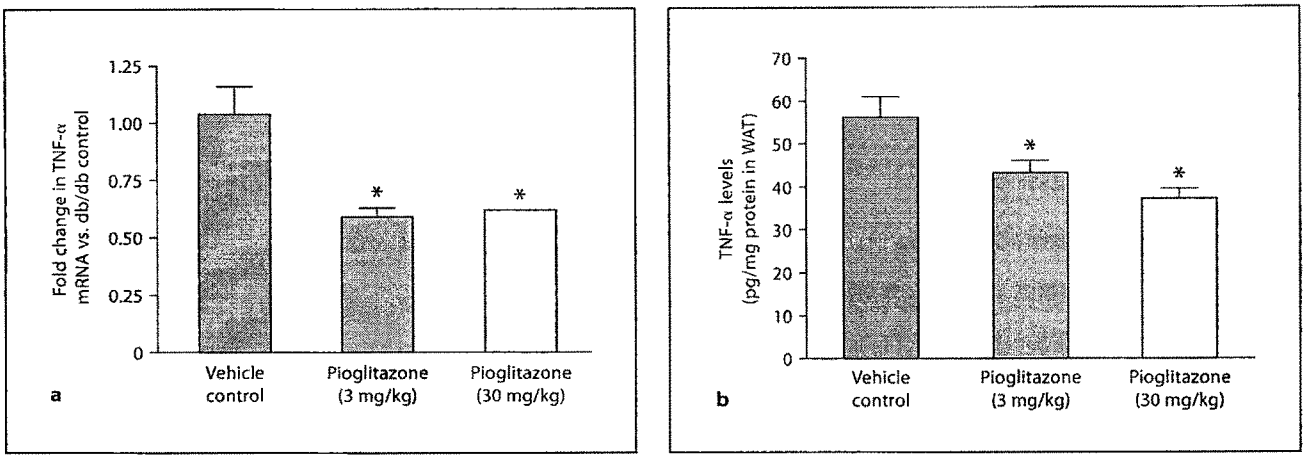


Fig. 5. a Expression of TNF-α mRNA in WAT of db/db mice was analyzed by quantitative real-time PCR. **b** TNF-α levels in WAT of db/db mice were analyzed by ELISA. Values are means ± SEM (n = 6 mice/group). * $p < 0.05$ compared to vehicle-treated control db/db mice.

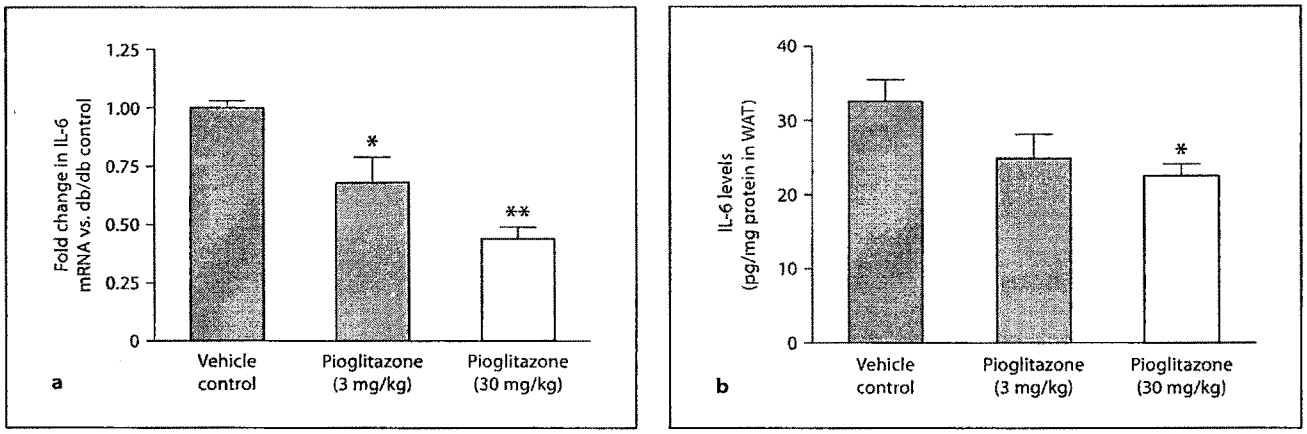


Fig. 6. a Expression of IL-6 mRNA in WAT of db/db mice was analyzed by quantitative real-time PCR. **b** IL-6 levels in WAT of db/db mice were analyzed by ELISA. Values are means ± SEM (n = 6 mice/group). * $p < 0.05$; ** $p < 0.01$ compared to vehicle-treated control db/db mice.

tazone treatment caused a significant reduction in TNF-α (43.12 ± 2.95 vs. 56.23 ± 4.78 pg/mg protein; n = 6; $p < 0.05$), but only slightly reduced IL-6 levels (24.9 ± 3.2 vs. 32.6 ± 2.91 pg/mg protein; n = 6) which were not significant when compared to the control group (fig. 5b, 6b). Resistin mRNA was found to decrease significantly ($p < 0.05$) in the 3-mg/kg dose group (fig. 7a). A significant lowering of serum resistin levels was observed at 3 mg/kg pioglitazone (7.40 ± 0.34 vs. 11.45 ± 0.85 ng/ml; n = 6; $p < 0.05$) which was in accordance with the tissue mRNA expression (fig. 7b).

The pioglitazone 30 mg/kg treatment decreased WAT TNF-α and IL-6 mRNA expression significantly when compared to the control animals. Interestingly, the reduction in TNF-α expression was found to be saturated at the low dose, and there was no significant difference between the 2 doses tested (fig. 5a, b). There was a significant reduction in IL-6 mRNA in both doses, whereas IL-6 protein levels decreased only at the higher dose. However, there was no statistical difference between the 2 dose groups of pioglitazone either in mRNA or protein levels (fig. 6a, b).

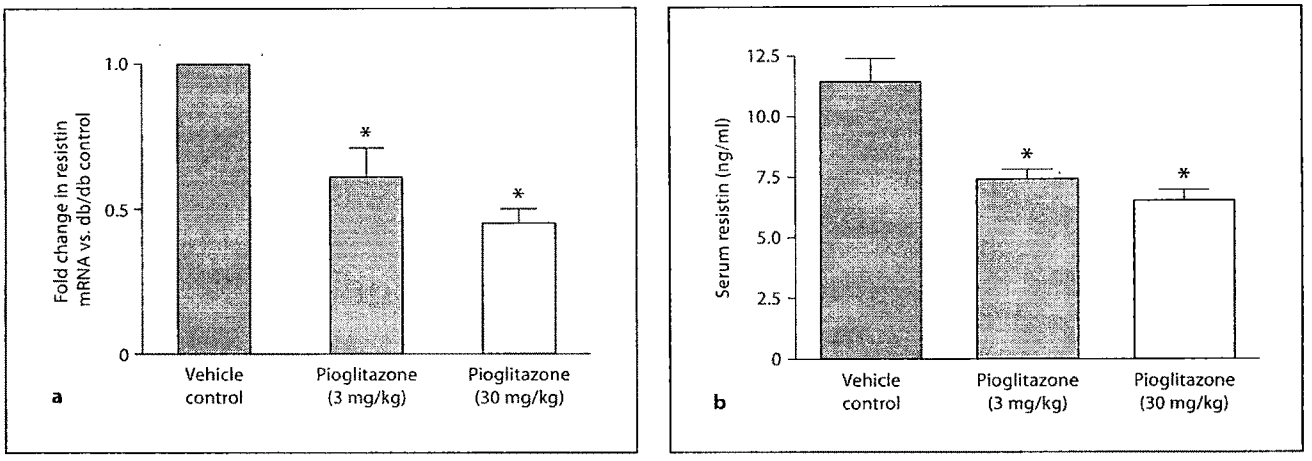


Fig. 7. **a** Expression of resistin mRNA in WAT of db/db mice was analyzed by quantitative real-time PCR. **b** Serum resistin levels in db/db mice were analyzed by ELISA. Values are means ± SEM (n = 6 mice/group). * $p < 0.05$ compared to vehicle-treated control db/db mice.

Discussion

In the present study, we tried for the first time to segregate the different effects of pioglitazone treatment at subtherapeutic and therapeutic doses using genetically altered mice expressing the phenotype of obesity and diabetes. At the therapeutic dose, pioglitazone demonstrated PPAR-γ-related changes by lowering the fasting glucose levels. An amelioration of hyperglycemia in the presence of reduced plasma insulin levels suggests that insulin sensitivity was improved in pioglitazone-treated db/db mice at the therapeutic dose. Our finding is supported by the fact that rosiglitazone significantly ameliorated hyperglycemia and hyperinsulinemia in diet-induced obese mice [30]. Along with the changes in glucose and insulin, there was also significant upregulation in the expression of some PPAR-γ-related metabolic markers, e.g. LPL, aP2 and adiponectin, in the WAT of db/db mice. In the present study, we observed that pioglitazone treatment at the therapeutic dose resulted in significant body weight gain in the db/db mice. This could be explained by the parallel increase in expression of the adipogenesis marker aP2 and LPL through PPAR-γ in the WAT. The increase of LPL synthesis in WAT may in part explain the increased adiposity observed during long-term treatment with TZDs [31, 32]. This observation was supported by reports from Yamauchi et al. [30] that rosiglitazone increased body weight in *db/db* mice and increased expression of adiponectin in differentiated 3T3L1 adipocytes in vitro. In our current study, the observed elevation of adiponectin could be explained as adiponectin is mainly expressed in adipocytes and is known to be affected by adipogenic differentiation. Therefore, our results indicate that a therapeutic dose of pioglitazone produces PPAR-γ-related changes in glucose levels and modulations of metabolic biomarkers in WAT.

However, treatment with a subtherapeutic (3 mg/kg) dose of pioglitazone did not alter serum glucose levels or other PPAR-related metabolic biomarkers (LPL, aP2 and adiponectin) in WAT, indicating the absence of metabolic effects of PPAR-γ. A similar observation was made by Srinivasan et al. [33] who demonstrated that a 3-mg/kg dose of pioglitazone was not sufficient to achieve antihyperglycemic and insulin-sensitizing effects in high-fat-fed rats. Interestingly, significant reductions in expression of TNF-α, IL-6 and resistin mRNA in WAT were found at this dose.

Reductions in TNF-α and IL-6 expression and in their corresponding protein levels in WAT at the subtherapeutic dose emphasize the anti-inflammatory effect of pioglitazone rather than its metabolic effect. Several reports indicated that TZDs may produce anti-inflammatory effects through nuclear factor-κB which is a molecular target for PPAR-γ agonists [25, 34, 35]. TNF-α and IL-6 are the important mediators of inflammation in preadipocytes and inhibit adipocyte differentiation in vitro [36]. Resistin is also considered as a proinflammatory cytokine [12, 37–39] and is known to inhibit adipogenesis [40]. It is also reported that proinflammatory cytokines/chemokines in preadipocytes suppress PPAR-γ and insulin responsiveness in

human adipocytes [41]. Low-grade systemic inflammation is associated with an increase in proinflammatory cytokines in obese condition [1, 2]. This is supported by the fact that an increased expression of TNF-α and IL-6 was observed in WAT of db/db mice compared to C57BL/6J mice (data not shown). The inhibition of TNF-α, IL-6 and resistin expression observed at the subtherapeutic dose of pioglitazone indicates that the drug may first correct the proinflammatory state of adipose tissue and may prime these tissues for insulin-sensitizing and adipogenic effects as indicated by the enhanced expression of adiponectin, LPL and aP2 at the therapeutic dose. This is supported by reports that the early anti-inflammatory role of pioglitazone or rosiglitazone in human diabetic patients is followed by delayed antidiabetic effects [29, 42].

In conclusion, our results indicate that the anti-inflammatory and insulin-sensitizing activities of pioglitazone are 2 distinctly separable effects. A subtherapeutic dose of pioglitazone produced anti-inflammatory effects by suppressing TNF-α and IL-6 in WAT without altering metabolic parameters. Thus, the anti-inflammatory effect precedes insulin-sensitizing effects observed at a therapeutic dose in diabetic db/db mice.

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References

1 Lion CJ, Law RE, Hsueh WA: Adiposity, inflammation, and atherogenesis. Endocrinology 2003;144:2195–2200.
2 Stuart P, Weisberg SP, McCann D, Desai M, Rosenbaum M, Rudolph L, Leibel RL, Ferrante AW Jr: Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003;112:1796–1808.
3 Fantuzzi G: Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol 2005; 115:911–919.
4 Antuna-Puente B, Feve B, Fellahi S, Bastard JP: Obesity, inflammation and insulin resistance: which role for adipokines. Therapie 2007;62:285–292.
5 Calabro P, Yeh ET: Obesity, inflammation, and vascular disease: the role of the adipose tissue as an endocrine organ. Subcell Biochem 2007;42:63–91.
6 Peraldi P, Xu M, Spiegelman BM: Thiazolidinediones block tumor necrosis factor-alpha-induced inhibition of insulin signaling. J Clin Invest 1997;100:1863–1869.
7 Rotter V, Nagaev I, Smith U: Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor-alpha, overexpressed in human fat cells from insulin-resistant subjects. J Biol Chem 2003;278:45777–45784.
8 Bastard JP, Maachi M, van Nhieu JT, Jardel C, Bruckert E, Grimaldi A, Robert JJ, Capeau J, Hainque B: Adipose tissue IL-6 content correlates with resistance to insulin activation of glucose uptake both in vivo and in vitro. J Clin Endocrinol Metab 2002;87: 2084–2089.
9 Xu H, Barnes GT, Yang Q, Tan G, Yang D, et al: Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 2003;112: 1821–1830.
10 Hotamisligil GS: Inflammatory pathways and insulin action. Int J Obes Relat Metab Disord 2003;27(suppl 3):S53–S55.
11 Silha JV, Krsek M, Skrha JV, Sucharda P, Nyomba BL: Plasma resistin, adiponectin and leptin levels in lean and obese subjects: correlations with insulin resistance. Eur J Endocrinol 2003;149:331–335.
12 Bokarewa M, Nagaev I, Dahlberg L, Smith U, Tarkowski A: Resistin, an adipokine with potent proinflammatory properties. J Immunol 2005;174:5789–5795.
13 Lago F, Dieguez C, Gómez-Reino J, Gualillo O: Adipokines as emerging mediators of immune response and inflammation. Nat Clin Pract Rheumatol 2007;3:716–724.
14 Chujo D, Yagi K, Asano A, Muramoto H, Sakai S, Ohnishi A, Shintaku-Kubota M, Mabuchi H, Yamagishi M, Kobayashi J: Telmisartan treatment decreases visceral fat accumulation and improves serum levels of adiponectin and vascular inflammation markers in Japanese hypertensive patients. Hypertens Res 2007;30:1205–1210.
15 Peng XD, Xie H, Zhao Q, Wu XP, Sun ZQ, Liao EY: Relationships between serum adiponectin, leptin, resistin, visfatin levels and bone mineral density, and bone biochemical markers in Chinese men. Clin Chim Acta 2008;387:31–35.
16 Tontonoz P, Hu E, Graves RA, Budavari AI, Spiegelman BM: mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. Genes Dev 1994;8:1224–1234.
17 Tontonoz P, Hu E, Spiegelman BM: Regulation of adipocyte gene expression and differentiation by peroxisome proliferator activated receptor gamma. Curr Opin Genet Dev 1995;5:571–576.
18 Rosen ED, Sarraf P, Troy AE, Bradwin G, Moore K, Milstone DS, Spiegelman BM, Mortensen RM: PPAR gamma is required for the differentiation of adipose tissue in vivo and in vitro. Mol Cell 1999;4:611–617.
19 Nolan JJ, Ludvik B, Beerdsen P, Joyce M, Olefsky J: Improvement in glucose tolerance and insulin resistance in obese subjects treated with troglitazone. N Engl J Med 1994; 331:1188–1193.
20 Tominaga M, Igarashi M, Daimon M, Eguchi H, Matsumoto M, Sekikawa A, Yamatani K, Sasaki H: Thiazolidinediones (AD-4833 and CS-045) improve hepatic insulin resistance in streptozotocin-induced diabetic rats. Endocr J 1993;40:343–349.
21 Mittra S, Sangle G, Tandon R, Sharma S, Roy S, Khanna V, Gupta A, Sattigeri J, Sharma L, Priyadarsiny P, Khattar SK, Bora RS, Saini KS, Bansal VS: Increase in weight induced by muraglitazar, a dual PPARalpha/gamma agonist, in db/db mice: adipogenesis/or oedema? Br J Pharmacol 2007;150:480–487.
22 Moore GBT, Chapman H, Holder JC, Lister CA, Piercy V, Smith SA, Clapham JC: Differential regulation of adipocytokine mRNA by rosiglitazone in db/db mice. Biochem Biophys Res Commun 2001;286:735–741.
23 Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, Nagaretani H, Matsuda M, Komuro R, Ouchi N, Kuriyama H, Hotta K, Nakamura T, Shimomura I, Matsuzawa Y: PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. Diabetes 2001;50:2094–2099.

24 Choi KC, Ryu OH, Lee KW, Kim HY, Seo JA, Kim SG, Kim NH, Choi DS, Baik SH, Choi KM: Effect of PPAR-alpha and -gamma agonist on the expression of visfatin, adiponectin, and TNF-alpha in visceral fat of OLETF rats. Biochem Biophys Res Commun 2005; 28:747–753.
25 Ghanim H, Garg R, Aljada A, Mohanty P, Kumbkarni Y, Assian E, Hamouda W, Dandona P: Suppression of nuclear factor-kappaB and stimulation of inhibitor kappaB by troglitazone: evidence for an anti-inflammatory effect and a potential antiatherosclerotic effect in the obese. J Clin Endocrinol Metab 2001;86:1306–1312.
26 Ghanim H, Dhindsa S, Aljada A, Chaudhuri A, Viswanathan P, Dandona P: Low-dose rosiglitazone exerts an antiinflammatory effect with an increase in adiponectin independently of free fatty acid fall and insulin sensitization in obese type 2 diabetics. J Clin Endocrinol Metab 2006;91:3553–3558.
27 Maegawa H, Nishio Y, Nakao K, Ugi S, Maeda K, Uzu T, Kashiwagi A: Short-term low-dosage pioglitazone treatment improves vascular dysfunction in patients with type 2 diabetes. Endocr J 2007;54:613–618.
28 Koufany M, Moulin D, Bianchi A, Muresan M, Sebillaud S, Netter P, Weryha G, Jouzeau JY: Anti-inflammatory effect of antidiabetic thiazolidinediones prevents bone resorption rather than cartilage changes in experimental polyarthritis. Arthritis Res Ther 2008;10: R6.
29 Forst T, Karagiannis E, Lübben G, Hohberg C, Schöndorf T, Dikta G, Drexler M, Morcos M, Dänschel W, Borchert M, Pfützner A: Pleiotropic and anti-inflammatory effects of pioglitazone precede the metabolic activity in type 2 diabetic patients with coronary artery disease. Atherosclerosis 2008;197:311–317.
30 Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T: The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 2001;7:941–946.
31 de Souza CJ, Eckhardt M, Gagen K, Dong M, Chen W, Laurent D, Burkey BF: Effects of pioglitazone on adipose tissue remodeling within the setting of obesity and insulin resistance. Diabetes 2001;50:1863–1871.
32 King AB, Armstrong DU: Lipid response to pioglitazone in diabetic patients: clinical observations from a retrospective chart review. Diabetes Technol Ther 2002;4:145–151.
33 Srinivasan K, Patole PS, Kaul CL, Ramarao P: Reversal of glucose intolerance by pioglitazone in high fat diet-fed rats. Methods Find Exp Clin Pharmacol 2004;26:327–333.
34 Chawla A, Barak Y, Nagy L: PPAR-gamma dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation. Nat Med 2001;7:48–52.
35 Diep QN, Amiri F, Benkirane K, Paradis P, Schiffrin EL: Long-term effects of the PPAR gamma activator pioglitazone on cardiac inflammation in stroke-prone spontaneously hypertensive rats. Can J Physiol Pharmacol 2004;82:976–985.
36 Lacasa D, Taleb S, Keophiphath M, Miranville A, Clement K: Macrophage-secreted factors impair human adipogenesis: involvement of proinflammatory state in preadipocytes. Endocrinology 2006;148:868–877.
37 Kaser S, Kaser A, Sandhofer A, Ebenbichler CF, Tilg H, Patsch JR: Resistin messenger-RNA expression is increased by proinflammatory cytokines in vitro. Biochem Biophys Res Commun 2003;309:286–290.
38 Lehrke M, Reilly MP, Millington SC, Iqbal N, Rader DJ, Lazar MA: An inflammatory cascade leading to hyperresistinemia in humans. PLoS Med 2004;1:e45.
39 Lu SC, Shieh WY, Chen CY, Hsu SC, Chen HL: Lipopolysaccharide increases resistin gene expression in vivo and in vitro. FEBS Lett 2002;530:158–162.
40 Kim KH, Lee K, Moon YS, Sul HS: A cysteine-rich adipose tissue-specific secretory factor inhibits adipocyte differentiation. J Biol Chem 2001;276:11252–11256.
41 Chung S, LaPoint K, Martinez K, Kennedy A, Sandberg MB, McIntosh MK: Preadipocytes mediate lipopolysaccharide-induced inflammation and insulin resistance in primary cultures of newly differentiated human adipocytes. Endocrinology 2006;147:5340–5351.
42 Mohanty P, Aljada A, Ghanim H, Hofmeyer D, Tripathy D, Syed T, Al-Haddad W, Dhindsa S, Dandona P: Evidence for a potent anti-inflammatory effect of rosiglitazone. J Clin Endocrinol Metab 2004;89:2728–2735.

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Research Paper

Involvement of adipokines in rimonabant-mediated insulin sensitivity in *ob/ob* mice

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Abstract

Objectives It has been recently reported that blockade of type 1 cannabinoid (CB1) receptors by specific antagonists or genetic manipulation alleviates dyslipidaemia, hyperglycaemia and insulin resistance in animal models of obesity and type 2 diabetes. However, the precise role of adipokines in the insulin-sensitising effects of the CB1 antagonist rimonabant is not clear.

Methods *ob/ob* mice were treated with different doses of rimonabant and then subjected to an oral glucose tolerance test. The expression of different adipokines in white adipose tissue was analysed by quantitative real-time PCR.

Key findings Rimonabant (30 mg/kg) significantly inhibited body weight and fat pad weight gain ($P < 0.05$) and improved glucose tolerance. Gene expression analysis indicated that tumour necrosis factor-α, visfatin and retinol binding protein-4 were downregulated in the adipose tissue of *ob/ob* mice treated with rimonabant compared with controls, whereas adiponectin was significantly upregulated.

Conclusions Rimonabant-mediated alteration of adipokines in white adipose tissues may play a role in improving insulin sensitivity in obese animals.

Keywords adipokine; adiponectin; cannabinoid receptor 1; retinol binding protein-4; tumor necrosis factor-α; visfatin

Introduction

The endocannabinoids have been implicated in the regulation of food intake and peripheral energy metabolism.[1,2] Higher endocannabinoid tone has been observed in adipose tissues of obese patients.[3] Cannabinoid type 1 (CB1) receptors are expressed in adipocytes[1,4–8] and appear to be upregulated in the adipose tissue of animals with genetically modified or diet-induced obesity.[8–10] CB1 receptor activation induces adipocyte differentiation, increases the activity of lipoprotein lipase and stimulates lipogenesis *in vitro*, while blockade of CB1 receptors by rimonabant prevents these effects,[9,10] indicating the role of CB1 receptors in adipocytes. Deletion of CB1 receptors leads to leanness and resistance to diet-induced obesity.[11] These experimental results suggest that the endocannabinoid system is crucial for understanding of obesity and associated metabolic syndrome. One striking feature of the clinical trial with a CB1 receptor antagonist was the improvement in insulin resistance found after 1 year of treatment, indicating a role of the endocannabinoid system in glucose homeostasis.[11] Activation of CB1 receptors induces glucose intolerance in rats and this is reversed by the selective CB1 receptor antagonist AM251.[12] Thus, CB1 antagonists may be useful in diabetes-associated obesity.

Although the pathophysiological mechanisms that underlie the metabolic syndrome are incompletely understood, insulin resistance appears to be an important component.[13] Adipokines such as adiponectin, leptin, tumour necrosis factor (TNF)-α and visfatin are thought to provide important links between obesity, insulin resistance and inflammatory disorders, including cardiovascular diseases.[14] Adiponectin reverses insulin resistance in a mouse model of lipodystrophy and obesity,[15] and CB1 antagonists have been reported to normalise or increase the expression of adiponectin mRNA and secretion of adiponectin from adipose tissue of obese mice as well as cultured 3T3-L1 adipocytes.[10] Visfatin,

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produced preferentially in visceral adipose tissue of mice and humans, binds to and activates the insulin receptor, exerting insulin-mimetic effects both *in vitro* and *in vivo*.[16] Retinol binding protein-4 (RBP-4) is a recently identified novel adipokine secreted from adipocytes. Several studies have found a correlation between serum RBP-4 levels and the magnitude of insulin resistance in human subjects with obesity, impaired glucose tolerance or type 2 diabetes.[17,18] Hotamisligil *et al.* reported a strong positive correlation between the expression of TNF-α mRNA in white adipose tissue (WAT) and the extent of hyperinsulinaemia in obese patients.[19] Both CB1 receptors and adipokines have been suggested to play important roles in insulin resistance and metabolic syndrome. However, the precise role of adipokines in the insulin-sensitising effects of CB1 receptor antagonists is still elusive. The purpose of the present study was to understand the involvement of adipokines in insulin sensitivity, mediated through CB1 receptor blockade. To examine this, the oral glucose tolerance test was performed in *ob/ob* mice treated with rimonabant and expression of RBP-4, adiponectin, TNF-α and visfatin in WAT was determined.

Materials and Methods

Animals

All animal experiments were carried out in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines, using protocols approved by the Institutional Animal Ethics Committee.

This study was performed in 8–10-week-old female *ob/ob* mice procured from the Jackson Laboratory (Bar Harbor, ME, USA). Mice were housed individually in ventilated cages and given pelleted food (Standard Rodent diet, NIN, Hyderabad, India) and water *ad libitum*, and were maintained at 25 ± 3°C and 50–70% humidity with a 12 h light–dark cycle.

Methodology

The animals were weighed and randomised into three groups of 12 each with similar mean body weight. The animals were given rimonabant, 3 or 30 mg/kg, or vehicle (0.5% v/v Tween 80) orally once daily for 14 days. On day 15, serum was collected. Half of the animals of each group were then anaesthetised, the abdomen opened and the epididymal, retroperitoneal, mesenteric and subcutaneous fat pads removed and weighed.[20] Samples of retroperitoneal WAT were flash frozen in liquid nitrogen for quantitative real-time PCR (qRT-PCR) analysis. Serum samples were stored at −70°C for later measurement of insulin and adiponectin. The remaining six animals in each group were subjected to an oral glucose tolerance test (OGTT), wherein an aqueous solution of glucose (3 g/kg in 10 ml) was administered orally after animals had been fasted for 18 h. Blood samples were taken via the retro-orbital sinus before (0 min) and 30, 60 and 120 min after glucose administration and glucose levels measured as described below.

RNA analysis and quantitative real-time PCR

Samples of WAT were homogenised in TRIzol reagent (Invitrogen, Life Technologies, Carlsbad, CA, USA) using a Mixer 301 (Retsch, Haan, Germany) and total RNA was extracted following the manufacturer's protocol. Then, 1 μg total RNA from each sample was taken for first-strand cDNA synthesis using a high-capacity cDNA archive kit (Applied Biosystems, Foster City, CA, USA). An equal amount of cDNA from each sample was taken for qRT-PCR using ABI-prism-7300 FAM-labelled Taqman probes viz. adiponectin, TNF-α and RBP-4 (all from Applied Biosystems). Taqman Universal Mastermix (Applied Biosystems) was used for expression profiling of the aforementioned target genes. The optimal primer concentration of visfatin for qRT-PCR was determined using the following combinations of forward and reverse primers: 50/50, 50/300, 50/900, 300/50, 300/300, 300/900, 900/50, 900/300 and 900/900 nmol/l. The concentration resulting in the lowest cycle threshold and best amplification efficiency was selected and used for qRT-PCR experiments (forward and reverse primers both 900 nmol/l). Amplification efficiency was determined by amplifying the different cDNA concentrations (10–100 ng) with the selected combination of forward and reverse primer. VIC-labelled mouse beta actin probe was co-amplified in each sample with every target gene(s) to normalise the results.

Serum measurements

Serum glucose levels were determined by the glucose oxidase/peroxidase (GOD/POD) method using a commercially available kit (Ranbaxy Laboratories, Gurgaon, India). Insulin (Linco Research Inc., St Charles, MO, USA) and adiponectin (B Bridge, Mountain View, CA, USA) levels in the serum were determined by ELISA according to the manufacturers' protocols.

Measurement of TNF-α in white adipose tissue

Samples of WAT were homogenised in ice-cold Tris buffer containing 1 mmol/l phenyl methyl sulfonyl fluoride for TNF-α estimation. TNF-α levels were measured by ELISA (BD Biosciences, San Jose, CA, USA) and were expressed per mg of tissue protein, measured using the biuret method (Pointe Scientific, Canton, MI, USA).

Statistical analysis

All values are given as means ± SEM. Statistical analysis of the data was done by one-way analysis of variance followed by Dunnett's multiple comparison test to identify differences between the groups. Difference was considered significant at $P < 0.05$. All analyses were performed using GraphPad software (version 4.0).

Results

Effect of rimonabant on body weight

Two weeks' treatment with 30 mg/kg rimonabant significantly decreased body weight gain in *ob/ob* mice compared with vehicle-treated animals (Figure 1). In parallel with reduction in body weight, subcutaneous and retroperitoneal fat pads were significantly reduced in weight in mice treated

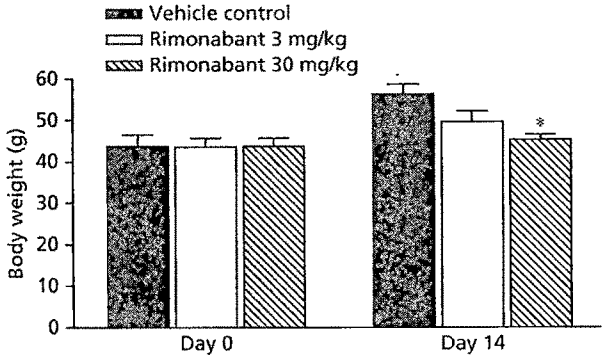

Figure 1 Effect of rimonabant on body weight in *ob/ob* mice. Values are means ± SEM (n = 12). $^*P < 0.05$ vs vehicle control on day 14.

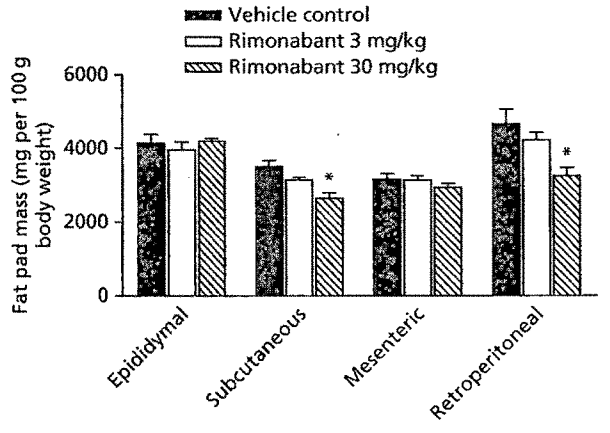

Figure 2 Fat pad weights in *ob/ob* mice treated with rimonabant for 14 days. Values are means ± SEM (n = 6). $^*P < 0.05$ vs vehicle control.

with 30 mg/kg rimonabant compared with control animals (Figure 2). Treatment with the 3 mg/kg dose slightly decreased body weight and fat pad weight but this was not significant when compared with control animals.

Effects of rimonabant on serum glucose, insulin and glucose tolerance

Rimonabant had a dose-dependent effect on the OGTT (data not shown) from which 30 mg/kg was identified as the optimal dose; 3 mg/kg showed no significant effect. As shown in Table 1, fasted serum glucose and insulin concentrations were significantly decreased in mice treated with rimonabant 30 mg/kg compared with control animals. However, no change in serum glucose or insulin was observed in the 3 mg/kg dose group. Serum glucose concentrations were significantly lower in the 30 mg/kg dose group than vehicle-treated animals at all time points after the oral glucose load (Figure 3). Based on the OGTT data, it was worth investigating how adipokines are modulated at these two dose levels of rimonabant.

Table 1 Effects of rimonabant on fasted serum glucose, insulin, adiponectin and tissue TNF-α levels after 14 days' treatment

	Rimonabant		
	Vehicle control	3 mg/kg	30 mg/kg
Glucose (mg/dl)	108.4 ± 3.9	95.7 ± 4.0	83.7 ± 2.6*
Insulin (ng/ml)	1.66 ± 0.11	1.56 ± 0.08	1.36 ± 0.04*
Adiponectin (μg/ml)	22.36 ± 0.66	24.1 ± 0.92	28.11 ± 0.88*
TNF-α (pg/mg)	86.23 ± 6.98	56.82 ± 3.95*	49.25 ± 2.67*

TNF-α, tumour necrosis factor α. Values are means ± SEM (n = 6). $^*P < 0.05$ vs vehicle control group.

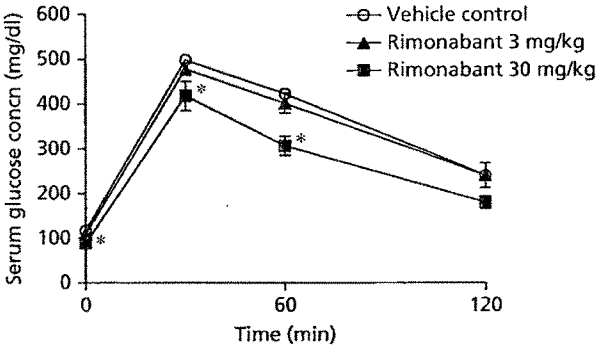

Figure 3 Effect of rimonabant treatment on the glucose tolerance test in *ob/ob* mice. Values are means ± SEM (n = 6). $^*P < 0.05$ vs vehicle-treated control *ob/ob* mice.

Effect of rimonabant on serum adiponectin and tissue TNF-α levels

Serum adiponectin level was significantly increased in mice treated with 30 mg/kg rimonabant. Serum TNF-α was found to be below the detectable limit so we measured TNF-α levels in adipose tissue. Rimonabant treatment caused a significant reduction in adipose TNF-α levels compared with the control group even at 3 mg/kg (Table 1).

Effect of rimonabant on the expression of adipokine genes in visceral adipose tissue

RBP-4 mRNA levels were significantly decreased by 30 mg/kg rimonabant compared with the vehicle control group (Figure 4a) whereas adiponectin mRNA levels were significantly increased at this dose (Figure 4b). The expression of adiponectin and RBP-4 mRNA in mice treated with 3 mg/kg rimonabant was not significantly different from that in control animals, although there was a tendency towards increasing mRNA levels of adiponectin and a decrease in RBP-4 in WAT. Expression of TNF-α and visfatin mRNA was significantly decreased after rimonabant treatment compared with the control group (Figure 4c and d).

Discussion

Blockade of CB1 receptors not only modulates feeding behaviour but also adipocyte biology and affects systemic glucose and lipid metabolism. Substantial data demonstrate

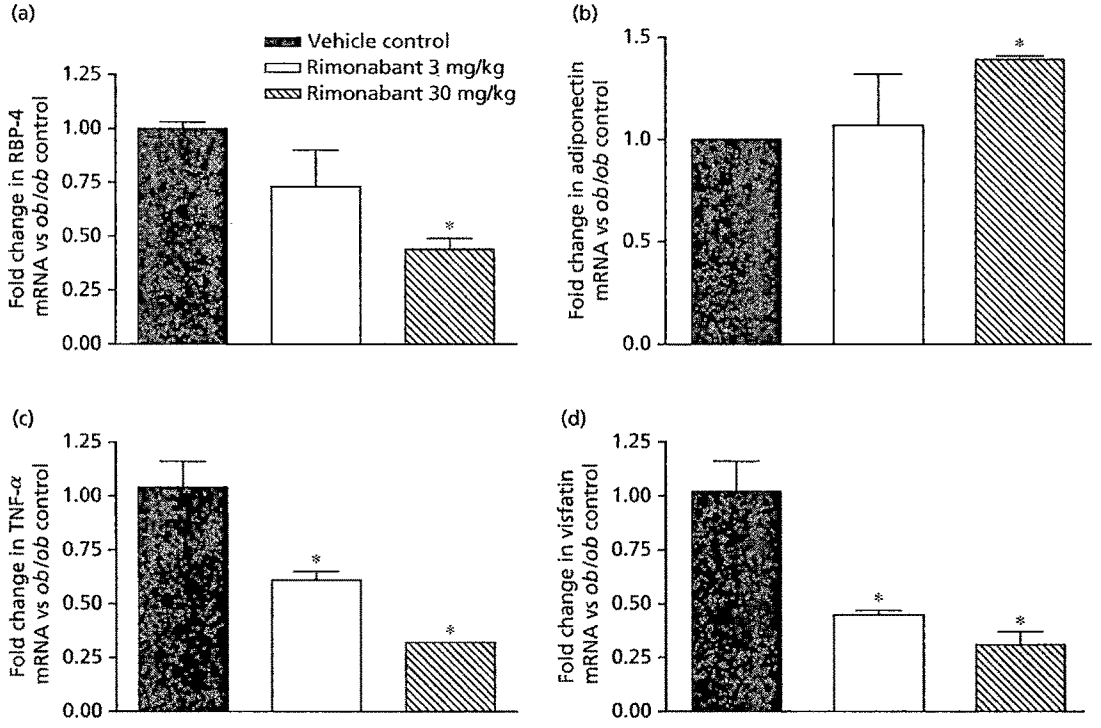

Figure 4 Expression of (a) RBP-4, (b) adiponectin, (c) tumour necrosis factor (TNF-)-α and (d) visfatin mRNA in white adipose tissue of *ob/ob* mice, determined by quantitative real-time PCR. The bars represent the fold change in the treatment groups compared with the vehicle control group, mean ± SEM (n = 6). *P < 0.05 vs untreated group.

the role of endocannabinoids on insulin resistance, and that CB1 blockade improves insulin sensitivity.[12] However, the precise role of adipose-derived cytokines in the insulin-sensitising effect of rimonabant is still not clear. In this study we have investigated the effect of rimonabant on insulin and WAT mRNA expression and serum levels of various adipokines in *ob/ob* mice, a model of obesity. Two weeks' treatment with rimonabant 30 mg/kg decreased body weight, fat pad weight, fasting plasma glucose and insulin and improved OGTT, which emphasises the insulin-sensitising effects of rimonabant. Trillou *et al.* reported similar findings in a mouse model of diet-induced obesity.[21]

To investigate the molecular mechanism of rimonabant-mediated insulin sensitivity, we examined RBP-4 expression in WAT. RBP-4 is overexpressed in WAT of adipose-specific GLUT4 knockout mice, which are insulin resistant, and underexpressed in WAT of transgenic mice over-expressing GLUT4 in adipose tissues, which have enhanced insulin sensitivity, suggesting that adipocyte-derived RBP-4 may act as an insulin resistance factor.[17] The expression and secretion of RBP-4 are positively regulated by insulin.[22] Higher RBP-4 levels found in diabetic patients were normalised by treatment with a thiazolidinedione.[23] In the present study, 2 weeks' treatment with rimonabant 30 mg/kg decreased WAT expression of RBP-4 mRNA in *ob/ob* mice. This is the first report of the effect of rimonabant on RBP-4 expression in rodents. It is possible that the decrease in RBP-4 expression by rimonabant may contribute to its insulin-sensitising effect.

To further explore the effects of rimonabant, we measured adiponectin mRNA in adipose tissue and its circulating levels. Our results showed that both adiponectin mRNA and circulating levels were upregulated by rimonabant at 30 mg/kg. Lower expression of adiponectin has been reported in obese individuals[24] and patients with type 2 diabetes.[25] Physiological doses of adiponectin improve insulin resistance in mouse models of obesity and type 2 diabetes.[26] In the present study, the observed increase in adipose expression of adiponectin after rimonabant treatment is in accordance with a previous study in Zucker fa/fa rats.[10] Elevated adiponectin expression correlated well with the levels in serum at the 30 mg/kg dose of rimonabant, which were in parallel with a fall in plasma glucose, insulin and improved glucose tolerance. Therefore, upregulation of adiponectin along with lowering of RBP-4 by rimonabant may lead to an improvement in insulin sensitivity. In a recent study, Lim *et al.* reported that exercise caused an increase in adiponectin and a decrease in RBP-4 levels and may lead to insulin sensitisation in young and middle-aged women.[27]

Overexpression of TNF-α is associated with increased adiposity and has been implicated in causing insulin resistance through inhibition of insulin receptor tyrosine kinase activity in adipose tissue.[19,28] We have observed significantly higher expression of TNF-α mRNA in obese *ob/ob* mice compared with lean C57BL/6J mice (data not shown). In the present study, 2 weeks' treatment with rimonabant lowered TNF-α mRNA expression and protein levels in visceral adipose tissue, even at the lower 3 mg/kg

dose. Similar reduction in lipopolysaccharide-induced serum TNF-α by rimonabant has been reported.[29] TNF-α inhibits insulin action in multiple ways – impairing phosphorylation of serine residues on insulin receptor substrate-1 (IRS-1) and activity of insulin receptor, and decreasing expression of IRS and GLUT-4.[28,30] A two-fold increase in insulin-stimulated tyrosine phosphorylation of the insulin receptor in the adipose tissue of TNF-α knockout mice has been reported, suggesting that insulin receptor signalling is an important target for TNF-α.[31] Inhibition of TNF-α by rimonabant may therefore improve insulin signalling.

Visfatin, another adipocytokine known to alleviate insulin resistance, exhibited an insulin-mimetic effect,[16] although Pagano *et al.* could not find a positive correlation between visfatin and insulin sensitivity.[32] The current study demonstrates for the first time that visfatin mRNA expression was significantly reduced after rimonabant treatment. Several reports describe visfatin as a new marker of inflammation. Visfatin expression is increased in different inflammatory conditions like atherosclerosis and inflammatory bowel disease.[33,34] In addition, visfatin expression is detected in synovial fibroblasts of patients with rheumatoid arthritis, and visfatin itself activates nuclear factor κB and related cytokines in cultured synovial fibroblasts.[35] The parallel suppression of visfatin and TNF-α by rimonabant even at a low dose emphasises its anti-inflammatory properties. Our data support previous finding of reduction in serum levels of RANTES and MCP-1 after long-term treatment with rimonabant in Zucker fa/fa rats.[36]

Global CB1 blockade enhances insulin sensitivity or glucose utilisation; however, blockade of central nervous system CB1 receptors did not improve insulin sensitivity, indicating involvement of peripheral CB1 receptors.[37] This is further supported by the increased glucose uptake of adipocytes *in vitro* following CB1 receptor stimulation.[7,38] Here it may be speculated that the improvement in insulin resistance produced by rimonabant is due to its direct effect on adipocytes to cause modulation of adipokines. However, further in-vitro studies are required to explain this phenomenon. These findings give a new insight into the development of peripheral CB1 antagonists as a possible target in the treatment of type 2 diabetes.

Conclusions

The insulin-sensitising effects of rimonabant in *ob/ob* mice may involve an increase in adiponectin, with concomitant decreases in expression of RBP-4 and TNF-α. Furthermore, our results indicate that rimonabant suppresses pro-inflammatory cytokines in adipose tissue, which may attenuate the low-grade inflammation due to excess adiposity and thus the metabolic syndrome.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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References

1. Cota D *et al.* The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *J Clin Invest* 2003; 112: 423–431.
2. Gonzalez-Yanes C *et al.* Oleylethanolamide impairs glucose tolerance and inhibits insulin stimulated glucose uptake in rat adipocytes through p38 and JNK MAPK pathways. *Am J Physiol Endocrinol Metab* 2005; 289: E923–E929.
3. Di Marzo V, Matias I. Endocannabinoid control of food intake and energy balance. *Nat Neurosci* 2005; 8: 585–589.
4. Steinberg BA, Cannon CP. Cannabinoid-1 receptor blockade in cardiometabolic risk reduction: safety, tolerability, and therapeutic potential. *Am J Cardiol* 2007; 100: 27–32.
5. Gary-Boboo M *et al.* The cannabinoid CB1 receptor antagonist rimonabant (SR141716) inhibits cell proliferation and increases markers of adipocyte maturation in cultured mouse 3T3 F442A preadipocytes. *Mol Pharmacol* 2006; 69: 471–478.
6. Matias I *et al.* Regulation, function, and dysregulation of endocannabinoids in models of adipose and beta-pancreatic cells and in obesity and hyperglycemia. *J Clin Endocrinol Metab* 2006; 91: 3171–3180.
7. Pagano C *et al.* The endogenous cannabinoid system stimulates glucose uptake in human fat cells via phosphatidylinositol 3-kinase and calcium-dependent mechanisms. *J Clin Endocrinol Metab* 2007; 92: 4810–4819.
8. Yan ZC *et al.* Exercise reduces adipose tissue via cannabinoid receptor type 1 which is regulated by peroxisome proliferator-activated receptor-d. *Biochem Biophys Res Commun* 2007; 354: 427–433.
9. Engeli SJ *et al.* Activation of the peripheral endocannabinoid system in human obesity. *Diabetes* 2005; 54: 2838–2843.
10. Bensaid M *et al.* The cannabinoid CB1 receptor antagonist SR141716 increases Acrp30 mRNA expression in adipose tissue of obese fa/fa rats and in cultured adipocyte cells. *Mol Pharmacol* 2003; 63: 908–914.
11. Van Gall L *et al.* Efficacy and safety of rimonabant for improvement of multiple cardiometabolic risk factors in overweight/ obese patients. *Diabetes Care* 2008; 31: S229–S240.
12. Bermúdez-Siva FJ *et al.* Activation of cannabinoid CB1 receptors induces glucose intolerance in rats. *Eur J Pharmacol* 2006; 531: 282–284.
13. Eckel RH *et al.* The metabolic syndrome. *Lancet* 2005; 365: 1415–1428.
14. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat Rev Immunol* 2006; 6: 772–783.
15. Kadowaki T *et al.* Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J Clin Invest* 2006; 116: 1784–1792.
16. Fukuhara A *et al.* Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science* 2005; 307: 426–430.
17. Yang Q *et al.* Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* 2005; 436: 356–362.
18. Graham TE *et al.* Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *N Engl J Med* 2006; 354: 2552–2563.
19. Hotamisligil GS *et al.* Increased adipose tissue expression of tumor necrosis factor-α in human obesity and insulin resistance. *J Clin Invest* 1995; 95: 2409–2415.

20. Remesar X *et al.* Effect of oral oleoyl-estrone on adipose tissue composition in male rats. *Int J Obes* 2002; 26: 1092–1102.
21. Trillou CR *et al.* Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-induced obese mice. *Am J Physiol Regul Integr Comp Physiol* 2003; 284: R345–R353.
22. Ost A *et al.* Retinol-binding protein-4 attenuates insulin-induced phosphorylation of IRS1 and ERK1/2 in primary human adipocytes. *FASEB J* 2007; 21: 3696–3704.
23. Hammarstedt A *et al.* High circulating levels of RBP4 and mRNA levels of aP2, PGC-1α and UCP-2 predict improvement in insulin sensitivity following pioglitazone treatment of drug-naïve type 2 diabetic subjects. *J Intern Med* 2008; 263: 440–449.
24. Hu E *et al.* AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem* 1996; 271: 10697–10703.
25. Statnick MA *et al.* Decreased expression of apM-1 in omental and subcutaneous adipose tissue of humans with type 2 diabetes. *Int J Exp Diabetes Res* 2000; 1: 81–88.
26. Yamauchi T *et al.* The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 2001; 7: 946–949.
27. Lim S *et al.* Insulin-sensitizing effects of exercise on adiponectin and retinol-binding protein-4 concentrations in young and middle-aged women. *J Clin Endocrinol Metab* 2008; 93: 2263–2268.
28. Hotamisligil GS *et al.* Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance. *Science* 1993; 259: 87–91.
29. Croci T *et al.* Role of cannabinoid CB1 receptors and tumor necrosis factor-a in the gut and systemic anti-inflammatory activity of SR 141716 (rimonabant) in rodents. *Br J Pharmacol* 2003; 140: 115–122.
30. Hotamisligil GS *et al.* IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-α and obesity-induced insulin resistance. *Science* 1996; 271: 665–668.
31. Hotamisligil GS. Mechanisms of TNF-α-induced insulin resistance. *Exp Clin Endocrinol Diabetes* 1999; 107: 119–125.
32. Pagano C *et al.* Reduced plasma visfatin/pre-B cell colony-enhancing factor in obesity is not related to insulin resistance in humans. *J Clin Endocrinol Metab* 2006; 91: 3165–3170.
33. Dahl TB *et al.* Increased expression of visfatin in macrophage of human unstable carotid and coronary atherosclerosis. *Circulation* 2007; 115: 972–980.
34. Moschen AR *et al.* Visfatin, an adipocytokine with pro-inflammatory and immunomodulating properties. *J Immunol* 2007; 178: 1748–1758.
35. Brentano F *et al.* Pre B cell colony enhancing factor/visfatin, a new marker of inflammation in rheumatoid arthritis with proinflammatory and matrix degrading activities. *Arthritis Rheum* 2007; 56: 2829–2839.
36. Schafer A *et al.* The cannabinoid receptor-1 antagonist rimonabant inhibits platelet activation and reduces pro-inflammatory chemokines and leukocytes in Zucker rat. *Br J Pharmacol* 2008; 154: 1047–1054.
37. Nogueiras R *et al.* Peripheral, but not central, CB1 antagonism provides food intake independent metabolic benefits in diet-induced obese rats. *Diabetes* 2008; 57: 2977–2991.
38. Gasperi V *et al.* Endocannabinoids in adipocytes during differentiation and their role in glucose uptake. *Cell Mol Life Sci* 2007; 64: 219–229.