Original Paper

Pioglitazone Lowers Serum Retinol Binding Protein 4 by Suppressing its Expression in Adipose Tissue of Obese Rats

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Key Words
Retinol binding protein 4 • Pioglitazone • Insulin resistance • Adipose tissue

Abstract
Background/Aims: Pioglitazone, a peroxisome proliferator-activated receptor γ activator, is clinically used to treat insulin resistance. However, the underlying mechanism of pioglitazone's action remains unclear. We investigated whether, and how, pioglitazone modulates serum level of retinol binding protein 4 (RBP4), an adipocytokine associated with obesity and insulin resistance. *Methods:* Insulin sensitivity was determined by oral glucose tolerance test, and RBP4 expression was detected by RT-PCR and Western blotting. *Results:* Pioglitazone treatment significantly decreased serum RBP4 levels in obese rats, which was correlated with reduced body weight and increased insulin sensitivity. Moreover, pioglitazone greatly decreased RBP4 mRNA and protein levels in adipose tissue but not in the liver. Consistently, pioglitazone treatment significantly reduced RBP4 protein expression in 3T3-L1 adipocytes but not in HepG2 cells. *Conclusion:* These results demonstrate that pioglitazone inhibits the level of serum RPB4 by suppressing RBP4 expression in adipose tissue of obese rats, suggesting that inhibiting RBP4 expression in adipocytes may provide a mechanism by which pioglitazone improves insulin sensitivity in insulin-resistant subjects.

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Introduction

Obesity and its metabolic disorders have been identified as a global health problem, and are strong risk factors for the development of type 2 diabetes mellitus by causing an insulin-resistant state in adipose tissue, liver, and muscle. Obesity is defined by abnormal or excessive lipid storage, and is characterized by increasing number and volume of adipocytes. Adipose tissue is not merely a depot for energy storage in the form of triglycerides (TG), but also regarded as a metabolically active endocrine organ which secrets numerous adipokines. The adipose tissue-secreted cytokines modulate inflammation and insulin resistance in diabetic patients [1]. Retinol binding protein 4 (RBP4) is a newly identified cytokine that is primarily secreted by adipocytes and hepatocytes, and is up-regulated during insulin resistance [2, 3]. High circulating RBP4 results in elevated blood glucose by increasing hepatic glucose output and inhibiting insulin signaling in skeletal muscle. An increasing body of evidence from both clinical and experimental studies have corroborated that elevated serum RBP4 level contributes to systemic insulin resistance and is associated with metabolic disorders, including diabetes, dyslipidemia, and cardiovascular risk factors [4-6]. Suppression of serum RBP4 by fenretinide, which increases urinary excretion of RBP4, improves insulin resistance and glucose intolerance [7, 8].

Thiazolidinediones (TZDs) is a class of pharmacological agents that ameliorate insulin resistance by enhancing proliferator-activated receptor γ (PPARγ) activity [9]. Activation of PPARγ improves insulin sensitivity by promoting fatty acid storage in adipocytes, inhibiting the production of inflammatory mediators and cytokines, and lowering blood glucose levels [10, 11]. Several studies have clearly shown that serum level of adipokine RBP4 is significantly down-regulated by TZDs treatment in obese mice and type 2 diabetic patients [12-14]. However, it remains unclear whether adipose tissue and/or the liver mediate suppression of serum RBP4 expression by PPARγ agonists. The aim of the present study was to address this question by examining the regulation of RBP4 in the liver and adipose tissue by pioglitazone, a PPARγ agonist that has anti-diabetic effects.

Materials and Methods

Animal treatment

Eight-week-old male Sprague-Dawley (SD) rats (180-220g, n = 24) were purchased from Sino-British Sippr/BK Lab Animal Ltd (Shanghai, China). After one week of quarantine (day 0), the rats were randomly assigned to either a standard chow diet group (CON, Slac Laboratory Animal Co. Ltd, China, 3.8 kcal/g, 55% of energy as carbohydrate, 21% as protein and 24% as fat) (n = 8), or a high fat diet group (HFD, 5.2 kcal/g, 20% of energy as carbohydrate, 21% as protein and 59% as fat) (n = 16). These diets contain sufficient amounts of vitamin A (20-25 IU/g). After eight weeks on HFD, half of the rats in the HFD group were given pioglitazone (Takada, Japan, dissolved in normal saline [0.9% NaCl], 20 mg/kg body weight (b.w.)/day) for four weeks by gavage as HFD pioglitazone-treated (HFD-PT) group, and the other half were given normal saline (0.9% NaCl) as HFD un-treated (HFD-UT) group. The chow diet group was also gavaged with saline. The pioglitazone dosage (20 mg/kg b.w./day) was determined as previously reported [15]. All animals had free access to diets and water under a controlled environment (19-22°C; 30-40% humidity, and 12 hours light/dark cycle). This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Shanghai Jiao Tong University. All surgeries were performed under sodium pentobarbital anesthesia and efforts were made to minimize animal suffering.

Insulin tolerance test (ITT)

ITT was performed at the end of pioglitazone or saline treatment by an I.P. injection of insulin (Humulin R, Eli Lilly and Company, USA) at 0.5U/kg b.w. after fasting for 4 h. Blood glucose concentration was determined with tail vein blood using a glucometer (Lifescan, Johnson-Johnson Medical (China) Ltd.). The blood glucose levels were measured at 0, 15, 30, 60, 90 and 120 min after insulin administration. The area under the curves for blood glucose (AUC_{ITT}) was calculated and used to estimate insulin sensitivity. AUC_{ITT} = (BG0'+BG120')/2+BG15'+BG30'+BG60'+BG90'.

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Oral glucose tolerance test (OGTT)

The animals were fasted overnight (12-16 hours) three days after ITT. The rats were given 2 g/kg b.w. of glucose through oral gavage. Blood was collected from the retro-orbital-sinus at 0, 30, 60 and 120 min after glucose challenge, and glucose and insulin levels were measured. The areas under the curves for blood glucose (AUC_{BG}) and insulin (AUC_{INS}) levels were calculated. AUC_{BG} = (BG0' + BG180')/2 + BG30' + BG60' + BG120', and AUC_{INS} = (INS0' + INS180')/2 + INS30' + INS60' + INS120'.

Blood biochemistry

Parameters of serum biochemistry were measured in overnight-fasted rats, including triglyceride, total cholesterol, high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), alanine aminotransferase (ALT), aspartate transaminase (AST), gamma glutamyl transpeptidase (GGT), and plasma glucose. The measurements were performed on a parallel multi-channel analyzer Glamour 2000 (MD Instruments, Muskegon,MI, USA). Serum RBP4 and insulin levels were measured with relevant ELISA kits (Phoenix Biotech, Beijing, China, and Millipore Corporation, Billerica, MA, respectively). The inter-assay coefficient of variation was less than 8 percent for RBP4 and 5 percent for insulin.

Cell culture and differentiation

3T3-L1 preadipocytes (American Type Culture Collection, Manassas, VA, USA)) were propagated and maintained in DMEM (Gibco, Grand Island, NY, USA) containing 10% (vol/vol) calf serum [13]. To induce differentiation, 2-day post-confluent preadipocytes at G1 phase (designated as day 0) were fed DMEM containing 10% (vol/vol) fetal bovine serum (FBS, Gibco, Grand Island, NY, USA), 1 µg/ml insulin, 1µM dexamethasone, and 0.5 mM 3-isobutyl-1-methylxanthine (IBMX,Sigma Aldrich, St.Louis, MO, USA) for two days. Cells were then cultured in DMEM supplemented with 10% FBS and 1µg/ml insulin for 2 more days, followed by incubation with DMEM containing 10% FBS. HepG2 cells were cultured in DMEM supplemented with 10% FBS and 100 µg/ml penicillin/streptomycin (Gibco, Grand Island, NY, USA). 3T3-L1 cells were treated with pioglitazone when over 90% of the cells had acquired adipose phenotype. Control cells received an equal volume of ethanol. HepG2 cells, at 70-80% confluence, were similarly treated after a 24-h starvation. Both adipocytes and hepatocytes were treated with pioglitazone at the concentration of 5, 10, or 20 µM, and cells were then collected for the analysis of RBP4 protein expression by Western blotting.

Reverse transcription PCR (RT-PCR)

Total RNA was isolated from the liver and subcutaneous adipose tissue using TRIzol (Invitrogen, Carlbad, CA, USA), and two µg of total RNA were reverse-transcribed into cDNA (Takara, Tokyo, Japan). In each RT-PCR reaction, 2 µl of cDNA was amplified in a final volume of 20 µl PCR reaction mixture using the TaqMan universal PCR master mix (Takara, Tokyo, Japan). Samples were incubated in the Perkin-Elmer PCR System 9700 (Applied Biosystems, Foster, CA, USA) for an initial denaturation at 95 ºC for 10 min, followed by 35 PCR cycles, with each cycle consisting of 95 ºC for 30 s, 62 ºC for 30 s, and 72 ºC for 40 s. The following primers were used: rat RBP4 (accession no. XM_215285.4) 5'- GACAAGGCTCGTTTCTCTGG -3' (sense) and 5'- AAAGGAGGCTACACCCCAGT -3' (antisense), and rat β-actin (accession no. NM_007393.1) 5'- CACGATGGAGGGGCCGGACTCATC -3' (sense) and 5'- TAAAGACCTCTATGCCAACACAGT -3' (antisense). The specificity of the PCR was further verified by subjecting the amplified products to agarose gel electrophoresis based on the expected product size. The mRNA levels of RBP4 and PPARγ were normalized to the internal control β-actin.

Western blotting

The liver and adipose tissues were homogenized in lysis buffer (20 mmol/L HEPES, pH 7.5, 150 mmol/L NaCl, 1 mmol/L EDTA, 1% Triton X-100, 0.5 mmol/L Na_3VO_4) with protease inhibitors (Roche Molecular Biochemicals, Mannheim, Germany). Cultured adipocytes and hepatocytes were lysed in the same lysis buffer, and crude homogenates were centrifuged at 12,000 rpm for 15 min at 4 ºC. Protein concentration was measured by BCA protein quantification Kit (Beyotime, Shanghai, China). Equal amount of proteins were separated on 10% SDS-PAGE gels, and then transferred onto nitrocellulose membranes (Millipore, Billerica, MA, USA). Membranes were blocked with 5% non-fat dried milk (NFDM) (Bright dairy, China) in TBST (Tris-buffered saline/Tween-20) for 1 h at room temperature, and then incubated with anti-RBP4 antibody (Abcam, UK) overnight at 4 °C. After three washes with TBST, 10 minutes each, membranes were

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incubated with HRP-labeled secondary antibody (Sigma, Sigma Aldrich, St.Louis, MO, USA) in 5% NFDM in TBST for 1 hour at room temperature and washed three times with TBST (10 minutes each). Signals were detected by chemiluminescence using the ECL detection reagent (Millipore, Billerica, MA, USA). The bands were scanned by Image Quant LAS 4000 mini (GE, Fairfield, CT, USA) and quantified by normalizing to the internal control, β-actin or β-tubulin.

Statistical analysis

All statistical analyses were performed with SPSS version 13.0 (Statistical Package for the Social Science, SPSS Inc., Chicago, IL). Results are expressed as means ± SD or SE. The analysis of Student's *t*-test or one-way ANOVA was used to identify significant differences between different groups. Spearman correlation was used to determine the main factor(s) that influence serum RBP4. All *P*-values are two-tailed and a *P*-value less than 0.05 is considered statistically significant.

Results

Pioglitazone reduces RBP4 expression, glucose and lipid metabolism in obese rats

We first explored whether pioglitazone improves glucose and lipid metabolism in HFD-fed obese rats. Rats fed a HFD showed a marked increase in body weight, fasting blood glucose, insulin and plasma lipid profiles including total cholesterol, triglyceride and LDL-C, and a decrease in HDL-C as compared to the normal diet fed control animals (Table 1). These metabolic changes were restored to normal levels by a 4-week pioglitazone treatment. Compared to HFD-UT group, the HFD-PT group elicited a significantly ($P < 0.05$) lower level of fasting blood glucose and insulin, triglyceride and LDL-C, and a significant higher level of HDL-C (Table 1). The expression level of ALT and GGT that was moderately elevated in the HFD-UT group was significantly ($P < 0.05$) attenuated by pioglitazone treatment (Table 1). Importantly, increase of serum RBP4 level in the HFD-UT group was abolished by poglitazone treatment in the HFD-PT group (Table 1).

Table 1. Effects of pioglitazone on body weight and metabolic values. The below parameters were analyzed with the blood from control (CON), HFD un-treated, and HFD pioglitazone-treated rats. HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, ALT: alanine aminotransferase, RBP4: retinol binding protein 4, AST: aspartate-aminotransferase, and GGT: γ-glutamyl-transpeptidase. Values are mean ± SE, and differences between groups were analyzed by t-test. a: compared with control group, and b: compared with HFD un-treated group. †: $P < 0.05$, ‡: $P < 0.01$

Parameters	CON ($n = 8$)	HFD	
		Un-treated ($n = 8$)	Pioglitazone-treated ($n = 8$)
Weight(g)	430 ± 32.6	500 ± 10.9 a‡	480 ± 17.1 b†
Fasting blood glucose (mM)	4.10 ± 0.19	5.45 ± 0.16 a‡	4.56 ± 0.35 b†
Fasting insulin (ng/ml)	0.35 ± 0.16	0.68 ± 0.09 a‡	0.45 ± 0.17 b‡
Triglyceride (mM)	0.63 ± 0.15	0.98 ± 0.31 a‡	0.72 ± 0.10 b†
Total cholesterol (mM)	1.16 ± 0.11	2.20 ± 0.27 a‡	1.29 ± 0.29 b†
HDL-C (mM)	0.69 ± 0.17	0.51 ± 0.20 a‡	0.60 ± 0.26
LDL-C (mM)	0.16 ± 0.08	0.31 ± 0.12 a‡	0.14 ± 0.10 b‡
ALT (u/l)	43.60 ± 7.60	57.40 ± 11.30 a†	46.10 ± 6.50 b†
AST (u/l)	206 ± 32	214 ± 55	196 ± 20
GGT (u/l)	2.00 ± 0.00	3.44 ± 3.04 a†	1.44 ± 0.52 b†
Serum RBP4 (μg/ml)	31.2 ± 8.2	78.3 ± 14.6 a‡	30.3 ± 8.0 b‡

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Fig. 1. Pioglitazone improved insulin sensitivity in obese rats. The values shown are (A) blood glucose and (B) serum insulin during oral glucose tolerance test (OGTT), and (C) blood glucose levels during insulin tolerance test (ITT). Data are presented the mean ± SD (n = 8). * P < 0.05, and ** P < 0.01.
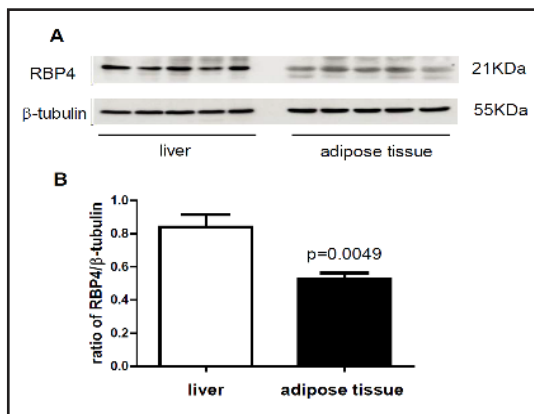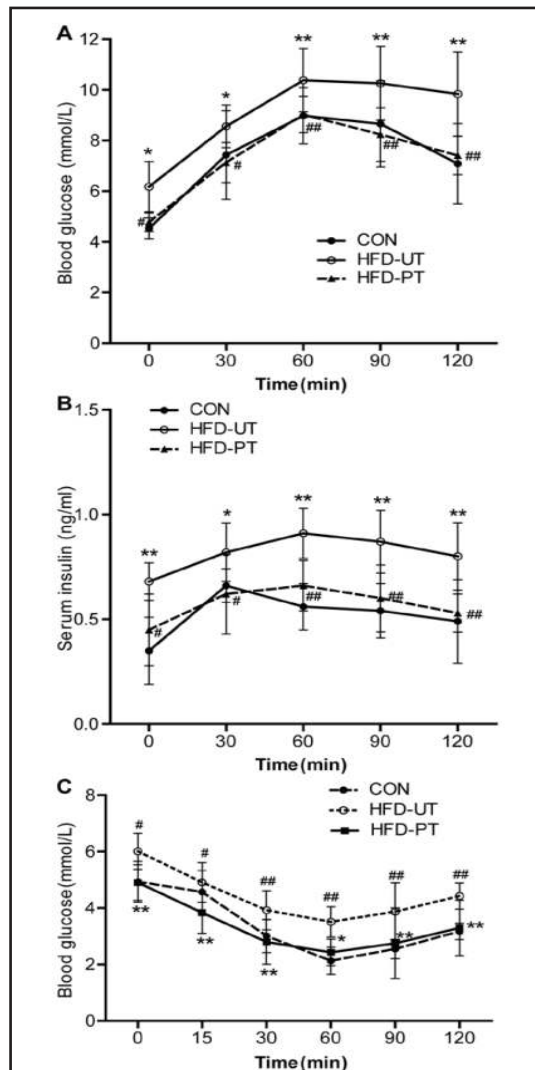

Fig. 2. The expression of RBP4 in the liver and adipose tissue of normal rats. Western blotting analysis shows that RBP4 expression in the liver was significantly (P < 0.05) higher than that in the adipose tissue.

Pioglitazone treatment improves insulin sensitivity in obese rats

We next examined whether pioglitazone improves insulin sensitivity in obese rats. Fasting insulin, AUC_{BG}, AUC_{INS} and AUC_{ITT} were measured to determine insulin sensitivity. All of the above indicators were higher in the HFD-UT group than in the control group (37.22 ± 4.71 vs. 29.45 ± 2.37; 3.34 ± 1.71 vs. 2.18 ± 1.07; 21.45 ± 2.07 vs. 15.39 ± 1.04; respectively, P < 0.01). These results suggested that insulin sensitivity was impaired in the HFD-UT rats. In contrast, fasting insulin levels (0.45 ± 0.17 ng/ml), AUC_{BG} (30.52 ± 2.42 mmol·min·L^{-1}), AUC_{INS} (2.37 ± 1.12 ng·min·ml^{-1}), and AUC_{ITT} (15.95 ± 2.14 mmol·min·L^{-1}) were all decreased in the HFD-PT group (Fig. 1).

Pioglitazone attenuates RBP4 expression in the adipose tissue of obese rats

Previous reports have demonstrated that RBP4 was primarily expressed in the liver and adipose tissue [16]. We herein assessed the expression of RBP4 in the liver and epididymis adipose of normal rats and showed that RBP4 expression was higher in the liver than in the adipose tissue (Fig. 2). Attenuation of serum RBP4 level (Table 1) by pioglitazone suggested that RBP4 expression might be down-regulated in the liver and/or adipose tissues. To determine the relative contribution of adipose tissue and liver to the serum RBP4 in response to pioglitazone treatment, we evaluated RBP4 expression at both the mRNA and protein levels in the adipose tissue and liver. The result showed that, compared to the control group, the HFD-UT group showed a significant (P < 0.01) increase in both RBP4 mRNA and protein
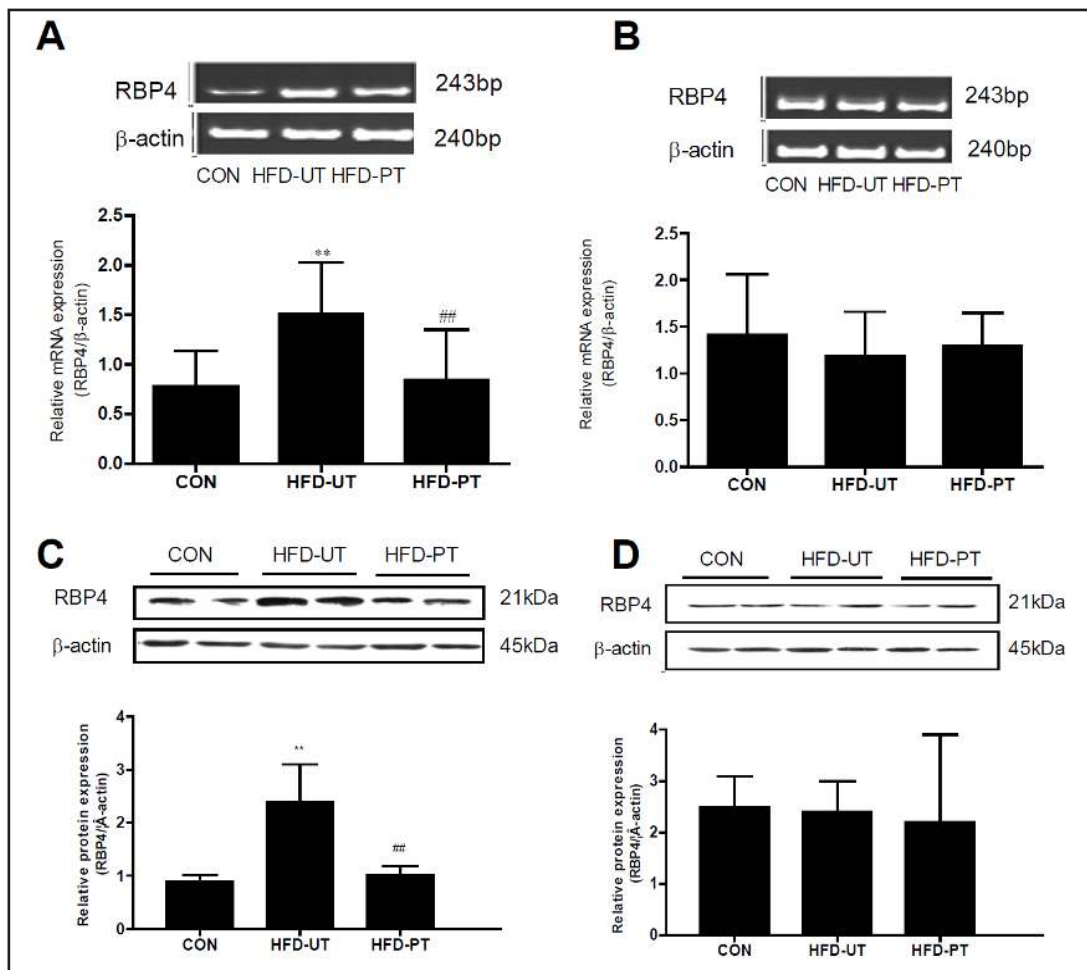
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Fig. 3. Pioglitazone attenuates obesity-associated increase of RBP4 expression in the rats. The expression of RBP4 mRNA (A and B) and protein (C and D) was examined by RT-PCR and Western blotting, respectively, in the adipose tissue (A and C) and liver (B and D) of control (CON), HFD un-treated (HFD-UT) and HFD pioglitazone-treated (HFD-PT) rats. The relative expression of RBP4 mRNA and protein was normalized to the internal control β-actin, and data are expressed as mean ± SE ($n = 8$) $^{**}P < 0.01$, compared with CON; and $^{##}P < 0.01$, compared with HFD-UT.

expression by 93.9% and 168%, respectively, in the epididymal adipose tissue (Fig. 3A and 3C). Moreover, the increased expression of RBP4 mRNA and protein were dramatically reduced by pioglitazone treatment in the HFD-PT group (Fig. 3A and 3C). In contrast, there was no significant difference in RBP4 expression at the mRNA or protein level in the liver among the control, HFD-UT, and HFD-PT groups (Fig. 3B and 3D). The difference of RBP4 expression in adipose tissue and liver of HFD-PT animals might be due to increase of PPARγ expression in adipose tissue, but not liver, in response to high fat feeding (data not shown). These findings suggest that changes of RBP4 gene expression in the adipose tissue, but not in the liver, might be responsible for fluctuation of serum RBP4 levels in response to HFD and pioglitazone treatment.

RBP4 expression in the adipose tissue positively correlates with insulin resistance
Serum RBP4 levels were positively correlated with AUC_{BG} ($R = 0.468, P < 0.05$) and AUC_{ITT} ($R = 0.636, P < 0.01$) (Table 2). Serum RBP4 levels were also significantly and positively correlated with RBP4 mRNA ($R = 0.594, P < 0.01$) and protein ($R = 0.648, P < 0.01$) levels in epididymal adipose tissue (Fig. 4A and 4B). By contrast, serum RBP4 was not correlated

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Fig. 4. Correlation of serum RBP4 with RBP4 expression in the adipose tissue and liver. Based on Spearman correlation analysis, serum RBP4 is significantly correlated with RBP4 expression at both mRNA (A, $r = 0.594$, $P < 0.01$) and protein (C, $r = 0.648$, $P < 0.01$) levels in the adipose tissues, but not with RBP4 expression at either mRNA (B, $r = 0.12$, $P = 0.57$) or protein (D, $r = 0.186$, $P = 0.358$) level in the liver.
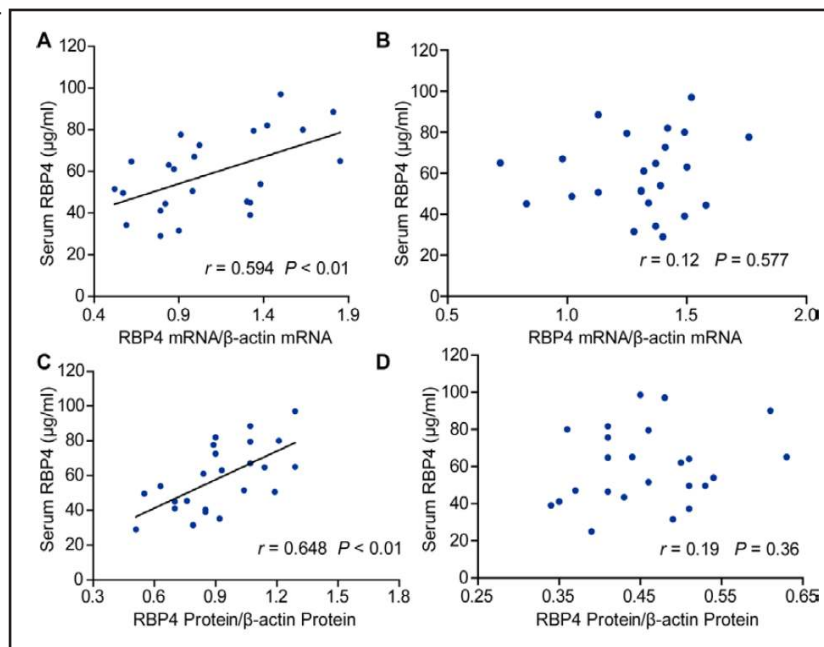

Table 2. Correlation of RBP4 levels with metabolic values. Spearman correlation analysis was conducted in the data pooled from all of the rats. ‡: $P < 0.01$, †: $P < 0.05$. NS, not significant correlation

	Serum RBP4	Fat		Liver	
		RBP4 mRNA	RBP4 protein	RBP4 mRNA	RBP4 protein
Triglyceride	0.430 †	0.515 ‡	0.580 ‡	NS	NS
Total cholesterol	0.501 ‡	0.371 †	0.523 ‡	NS	NS
LDL-c	0.451 †	0.329 †	0.308 †	NS	NS
HDL-c	-0.449 †	NS	-0.362 †	NS	NS
Fasting blood glucose	0.510 ‡	NS	0.608 ‡	NS	NS
Fasting insulin	0.535 ‡	0.568 ‡	0.683 ‡	NS	NS
AUC$_{BG}$	0.468 †	0.609 ‡	0.527 ‡	NS	NS
AUC$_{ITT}$	0.636 ‡	0.615 ‡	0.500 ‡	NS	NS

with RBP4 expression at either mRNA (R = 0.12, $P = 0.57$) or protein (R = 0.186, $P = 0.358$) level in the liver (Fig. 4C and 4D). Additionally, both the mRNA and protein expression of RBP4 in the adipose tissue were positively correlated with AUC$_{BG}$ (R = 0.609 and R = 0.527, respectively, both $P < 0.01$) and AUC$_{ITT}$ (R = 0.615 and R = 0.500, respectively, both $P < 0.01$). However, there was no significant correlation between RBP4 expression in the liver and insulin resistance relevant parameters (Table 2). Moreover, we found that serum RBP4 and RBP4 expression in the adipose tissue but not in the liver were correlated with the metabolic data such as triglyceride, total cholesterol and LDL-C (Table 2). Taken together, these data indicate that pioglitazone ameliorates insulin resistance by down-regulating the expression of RBP4 in adipose tissue of obese rats.

Pioglitazone down-regulates RBP4 expression in cultured 3T3-L1 adipocytes
    We further confirmed whether the inhibitory effect of pioglitazone on RBP4 expression was reproducible in cultured adipocytes using hepatocytes as a control. Cultured 3T3-L1 adipocytes and HepG2 hepatocytes were first exposed to 300 μM palmitate for 24h, which was followed by a combined treatment with pioglitazone at the concentration of 5, 10, or
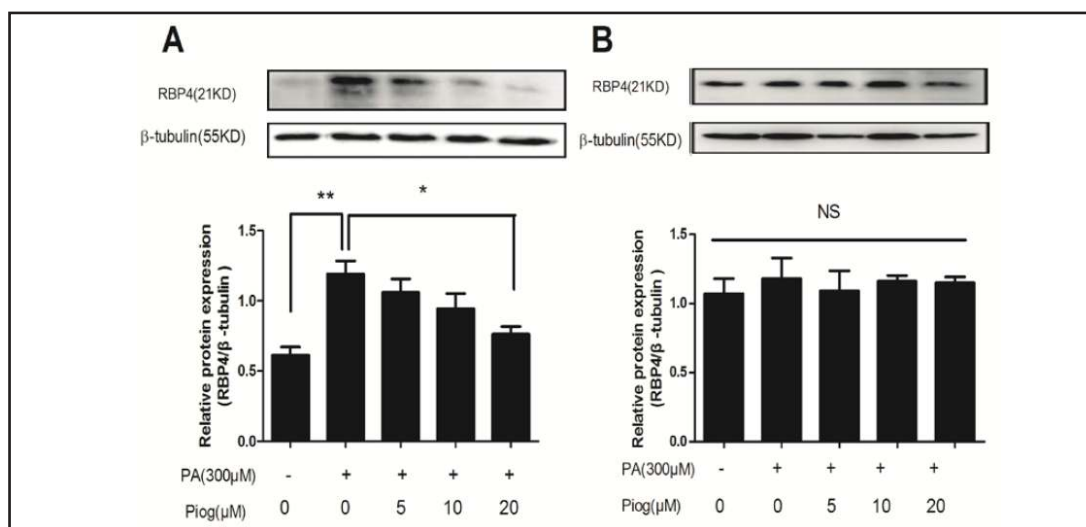
Fig. 5. Pioglitazone attenuates RBP4 expression in 3T3-L1 adipocytes but not HepG2 hepatocytes. Both 3T3-L1 adipocytes (A) and HepG2 hepatocytes (B) were treated with 300 M palmitic acid (PA) for 24h, with or without pioglitazone (Piog) at the concentration of 5, 10, or 20 μM. The relative expression of RBP4 protein was normalized to the internal control β-tubulin and data are expressed as mean ± SE ($n = 4$). $^{**}P < 0.01$, $^{*}P < 0.05$, and NS, not significant.

20 μM. It is important to note that cell viability test showed no cytotoxicity to either 3T3-L1 or HepG2 cells with 300 μM palmitate (data not shown). Palmitate treatment increased protein expression of RBP4 in 3T3-L1 adipocytes by 100% (Fig. 5A). Consistent with our findings in *in vivo* adipose tissue, pioglitazone at 20 μM suppressed RBP4 protein level by approximately 44% in 3T3-L1 adipocytes, whereas pioglitazone at lower concentrations did not cause a significant change despite of a slight decrease. On the other hand, neither palmitate nor pioglitazone altered the expression of RBP4 in HepG2 cells (Fig. 5B). These results demonstrate that pioglitzone specifically regulates RBP4 expression in the adipocytes, but not in the hepatocytes.

Discussion

With an increasing prevalence of obesity, there is a trend toward a higher incidence of insulin resistance, hyperlipidemia, cardiovascular diseases, type 2 diabetes and fatty liver. Adipocyte-derived cytokines have been proposed as diagnostic markers and therapeutic targets for obesity-linked insulin resistance, diabetes, and fatty liver [17]. Many adipokines are implicated directly in the pathologies associated with obesity, particularly the metabolic syndromes [18]. RBP4 is a compact and globular protein that was first identified as a retinol carrier, and is mainly synthesized and secreted by the liver [19]. Recently, an increasing body of evidence has identified RBP4 as an adipokine and elevated RBP4 expression is closely related to insulin resistance and type 2 diabetes [20]. Ost et al. showed that RBP4 is likely released from the adipocytes and acts locally to inhibit phosphorylation of IRS1 at serine 307, thereby disrupting insulin-mediated nutrient sensing [21]. In the present study, we showed that serum RBP4 level in rats is enhanced by high fat diet and that RBP4 is primarily synthesized in the liver and secondly in the adipose tissue. We further showed that pioglitazone treatment attenuated circulating RBP4 abundance in obese rats. Pioglitazone is a selective and potent ligand for the nuclear transcription factor PPARγ. It has been widely used to ameliorate hyperglycemia and to improve insulin sensitivity in type 2 diabetic patients. The role of pioglitazone in the treatment of obesity and insulin resistance

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insulin is through the control of glucose and lipid metabolism in the liver, adipose tissue and muscle by modulating the transcription of -sensitive genes [22]. Our finding of the inhibitory effect of pioglitazone on serum RBP4 expression is consistent with a previous report that pioglitazone improves insulin resistance through decreasing the level of serum RBP4 [23].

The primary goal of this study was to understand the main source of RBP4 secretion that contributes to the change of serum RBP4 level under obese/diabetic and therapeutic conditions. We demonstrated for the first time that high fat diet-induced increase and pioglitazone-mediated decrease in the abundance of circulating RBP4 are primarily due to change of RBP4 expression in the adipose tissue. These findings are consistent with our previous report that fenofibrate, a PPARα agonist, suppresses serum RBP4 level also by down-regulating RBP4 expression in the adipose tissue [24]. However, Yao-Borengsser *et al.* showed that pioglitazone increased RBP4 gene expression in the white adipose tissue of human patients [25]. This discrepancy may be due to the analysis of RBP4 expression in different adipose tissues: visceral adipose tissue in this study while subcutaneous adipose tissue in the human patients' study. Surprisingly, we did not find a change of RBP4 expression in the liver in response to either high fat diet or pioglitazone treatment, although RBP4 is abundantly expressed in the liver and secreted into the circulation in a 1:1:1 complex with retinol (holo-RBP4) and transthyretin [26]. This finding from the *in vivo* tissues was confirmed by the *in vitro* cell culture experiment wherein pioglitazone inhibited RBP4 expression only in adipocytes, but not hepatocytes. Our observation may help to explain a previous finding by Bahr *et al.* that hepatic RBP4 expression is not associated with insulin resistance [27]. However, it remains unknown why RBP4 expression is differentially regulated by pioglitazone in the adipose tissue and liver. One explanation may be related to the expression of PPARγ. PPARγ, the downstream effector of pioglitazone in RBP4 regulation, is more abundantly expressed in the adipose tissue than in the liver. In fact, previous studies have shown that PPARγ is predominantly expressed in adipose tissues and immune system and, to a lesser extent, in the liver [28].

In conclusion, we showed that serum RBP4 was increased in obese rats and pioglitazone treatment suppressed the abundance of circulating RBP4. RBP4 expression was higher in the liver and relatively lower in the adipose tissue; however, only RBP4 in the adipose tissue was subjected to regulation in obese condition and by the PPARγ activator, pioglitazone. These findings suggest that targeting RBP4 expression in the adipose tissue is a potential strategy in the treatment of insulin resistance and type 2 diabetes.

Abbreviations

RBP4 (retinol binding protein 4); PPAR (peroxisome proliferator-activated receptor); HDL-C (high-density lipoprotein cholesterol); LDL-C (low-density lipoprotein cholesterol); ALT (alanine aminotransferase); AUC_{BG} (areas under curves of glucose); AUC_{INS} (areas under curves of insulin); AUC_{ITT} (areas under curves of glucose in insulin tolerance test); AST (alanine aminotransferase); GGT (γ-glutamyl-transpeptidase).

Acknowledgment

Disclosure Statement

There are no conflicts of interest for the study.

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