

Original Article

Effects of chronic ethanol consumption on levels of adipokines in visceral adipose tissues and sera of rats

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Aim: To investigate the effects of ethanol on adipokines (leptin, adiponectin, resistin, visfatin and cartonectin) levels in visceral adipose tissue (VAT) and sera, and explore the correlation between VAT and serum adipokine levels.

Methods: Forty-eight Wistar rats were randomly divided into control, low, middle and high ethanol treatment groups that received 0, 0.5, 2.5, or 5.0 g of ethanol/kg¹·d¹, respectively, via gastric tubes for 22 weeks. The levels of fasting blood glucose (FBG) and fasting serum insulin (FINS) were measured and homeostasis model assessment of insulin resistance (HOMA-IR) values were calculated. Adipokines in perirenal and epididymal VAT and sera were measured by enzyme-linked immunosorbent assays (ELISAs).

Results: High-dose treatments of ethanol (vs control group) significantly increased FINS (eg 37.86%) and HOMA-IR values (eg 40.63%). In VAT, levels of leptin, resistin and visfatin in the middle- and high-dose groups were significantly elevated, whereas adiponectin and cartonectin levels decreased. In sera, changes in adipokine levels were similar to that observed in VAT, with the exception of cartonectin. These ethanol-induced effects were dose-dependent. A positive correlation existed between VAT and serum adipokine levels, except for cartonectin.

Conclusion: Chronic ethanol consumption affects adipokine levels in VAT and sera in a dose-dependent manner, with the exception of serum cartonectin. The altered levels of adipokines in VAT and sera are positively correlated.

Keywords: ethanol; adipokines; visceral adipose tissue; serum

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Introduction

Both white and brown adipose tissue (WAT and BAT, respectively) can be distinguished histologically and functionally. Whereas BAT is specialized in the production of heat, WAT stores excess energy as triacylglycerols. Recent studies demonstrate that WAT is an important endocrine organ that produces numerous peptides and proteins with broad biological activity^[1]. Secretory products of WAT are collectively called adipokines. Among these adipokines, adiponectin, leptin, resistin and visfatin are implicated in the development of metabolic syndrome^[2]. Leptin is considered one of the main peripheral endocrine signals involved in the regulation of appetite and body weight^[3]. Adiponectin is a potent anti-

inflammatory and anti-diabetic protein. In contrast, resistin causes — at least in the murine system—insulin resistance. Visfatin is highly enriched in the visceral adipose tissue of both humans and rodents. It mimics the effects of insulin by lowering plasma glucose levels. Cartonectin is an adipocyte secretory protein that was identified in 2001^[4]. It is an adiponectin paralog and a member of the C1q/TNF- α molecular superfamily, which not only has anti-inflammatory properties^[5] but also can effectively stimulate adiponectin and resistin secretion from murine adipocytes *in vitro*^[6]. This stimulatory property of cartonectin demonstrates that it is a potentially important adipokine in the diabetes research field.

Recent studies reported that both subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) are metabolic risk factors. However, VAT is a more potent adverse metabolic risk agent than SAT because it is strongly linked with type 2 diabetes, hypertension and dyslipidemia. Basat *et al* found that the amount of VAT is significantly higher in non-obese patients with new onset type 2 diabetes than in healthy, non-

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diabetic patients^[7-9]. Thus, some experts consider VAT levels important indicators of insulin resistance. Based on this line of evidence, adipokine measurements from VAT should facilitate a more complete understanding of the molecular mechanisms of metabolic syndrome, such as insulin resistance.

At present, ethanol is the most widely abused addictive substance. In addition to decreasing glucose uptake in rat adipocytes^[10], chronic ethanol exposure in rats has been shown to increase the rate of triglyceride degradation in adipose tissue, resulting in elevated circulating free fatty acids (FFAs)^[11]. Our prior results measured depressed insulin-stimulated glucose uptake and increased insulin resistance in isolated skeletal muscle of rats exposed to chronic excess ethanol^[12]. We also observed down-regulated expression of the glucose transporter, GLUT4, in rat cardiac muscle tissue^[13]. However, how is the effect of chronic ethanol feeding on adipokines?

Previous studies investigating the effects of ethanol on adipokines have concentrated mainly on their detection levels in serum. Due to different experimental conditions, no consistent conclusions can be drawn concerning the effects of ethanol on adipokines^[14]. Most studies report that ethanol elevated serum levels of leptin and resistin. Other studies indicate that serum leptin levels in rodents and humans were decreased or unchanged with ethanol intake and that resistin levels in human serum were not affected by moderate ethanol intake. Serum adiponectin levels decreased in rats chronically treated with high doses of ethanol, while rats treated with moderate doses of ethanol had elevated adiponectin levels. Nevertheless, data are sparse regarding the effects of ethanol on adipokine levels in adipose tissue. In addition, to the best of our knowledge, no previous studies have described the effects of ethanol on visfatin and cartonectin levels.

Therefore, the objectives of the present study were to determine VAT and sera levels of leptin, adiponectin, resistin, visfatin and cartonectin in rats exposed to ethanol treatments using enzyme-linked immunosorbent assays (ELISAs) and to explore correlations between these VAT and serum adipokine levels.

Materials and methods

Animals

Forty-eight male, specific pathogen free (SPF), Wistar rats (180–200 g; 8 weeks old) were purchased from the Experimental Animal Center, Shandong University, China. After acclimatization for one week, rats were randomly allocated into the following four experimental groups ($n=12$ per group): control group (distilled water at $5.0 \text{ g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$), low-dose group (ethanol at $0.5 \text{ g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$), middle-dose group (ethanol at $2.5 \text{ g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$) and high-dose group (ethanol at $5.0 \text{ g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$). Distilled water or edible ethanol (50% *v/v*; Ji-nan Baotu Spring Distillery, Shandong, China) was delivered by gastric tube once daily between 8:00 and 9:00 AM. Rat body weights were monitored and ethanol volumes were adjusted weekly. All treatments lasted for 22 weeks.

During treatments, all rats were housed in individual cages under constant temperature (25 °C) and humidity (50%). Food

and water were available *ad libitum* under a 12 h light/12 h dark cycle. Food was purchased from the Laboratory Animal Center, Shandong University (Ji-nan, China). The animal study was approved by the Shandong University Institutional Animal Care and Use Committee.

Specimen collection and storage

After a 22-week treatment, rats fasted overnight and were anesthetized by intraperitoneal injection of 3% sodium pentobarbital (0.1 mL/100 g BW). Blood samples (6–8 mL) were obtained from the inferior vena cava, and sample aliquots were placed in procoagulant tubes. The supernatant was acquired by centrifugation and allocated to distinct EP tubes, after which it was stored at -80 °C. Epididymal and perirenal fat pads were quickly dissected and weighed to calculate the relative adipose tissue weight to body weight ratio. Tissues were frozen in liquid nitrogen for adipokine analyses.

Biochemical analysis and evaluation of insulin sensitivity

Fasting blood glucose (FBG) levels were measured by the glucose oxidase method, and fasting serum insulin (FINS) levels were measured by radioimmunoassay (RIA; Northern Bioengineering Institute, Beijing, China). The homeostasis model assessment of insulin resistance (HOMA-IR) value was calculated by the following formula: $\text{FBG (mmol/L)} \times \text{FINS } (\mu\text{mol/mL}) / 22.5^{[15]}$.

Extraction of total proteins in adipose tissues

Frozen adipose tissues were placed on ice, and 200-mg excisions were taken from these frozen samples. The excised tissues were dissolved in 200 μL PBS (0.01 mol/L; pH 7.4) and homogenized using a VCX450 Ultrasonic Cell Disruption System (Time: 30 s; Temp: 4 °C; Pulse on: 3 s, Pulse off: 5 s; Amplifier: 28%–30%; Xinzhi Biotechnology Co, Ltd, Ningbo, China). Adipose tissue homogenates were frozen overnight at -80 °C and thawed on ice the following day. After two freeze-thaw cycles, homogenates were centrifuged with a High Speed Refrigerated Centrifuge (Beckman, USA) at 15000 r/min for 30 min (4 °C). The middle layer was the protein-required liquid.

Enzyme-linked immunosorbent assays (ELISAs)

The protein concentrations extracted from adipose tissues were determined with a Protein Assay Kit (BCA, Bio-Rad, Hercules, CA, USA) and bovine serum albumin (BSA) was used as a standard. After being diluted to 1 $\mu\text{g}/\mu\text{L}$ with PBS (0.01 mol/L; pH 7.4), protein isolations were aspirated (100 $\mu\text{L}/\text{sample}$) to detect leptin, resistin, visfatin and cartonectin. The protein isolation for the adiponectin assay was not diluted.

Concentrations of leptin, adiponectin, resistin, visfatin and cartonectin in both adipose tissues and sera were measured with ELISA kits (leptin, resistin and visfatin: Usnlife Science & Technology Co, Ltd, Wuhan, China; adiponectin: Bionewtrans Pharmaceutical Biotechnology Co, Ltd, Franklin, USA; cartonectin: Blue Gene Biotechnology Co, Ltd, Shanghai, China). The detection of all adipokines was performed accord-

ing to the manufacturer's instructions. Serum levels of TNF- α were measured according to the manufacturer's instructions using a TNF- α assay kit (R&D systems, Minneapolis, USA).

Statistical analysis

All values are reported as mean \pm SD. Data were analyzed using SPSS 11.5 software (SPSS, Inc, Chicago, IL, USA). The LSD statistical test was used for a *post hoc* comparison after the ANOVA. Simple linear correlation analysis between two variables was applied. $P < 0.05$ was considered statistically significant.

Results

Chronic ethanol consumption raised insulin resistance index

FBG levels in rats increased compared to the control group but these changes were not statistically significant. High doses of ethanol increased FINS levels by 37.86% ($P < 0.05$) and HOMA-IR values by 40.63% ($P < 0.05$) compared to the control group respectively. These increases indicate that chronic ethanol consumption raised the insulin resistance index, especially in rats receiving high doses of ethanol.

Four treatment groups, including ethanol treatments and control, had similar body weight (BW) at baseline. After the 22-week treatment, the BW of the high-dose group decreased by 17.56% ($P < 0.01$) but the epididymal adipose tissue weight (epididymal adipose tissue weight/BW) decreased by 33.33% ($P < 0.05$) in relation to the control group. The variances in low- and middle-dose ethanol treatments did not change (Table 1).

Chronic ethanol treatments increased leptin in VAT and sera

Chronic ethanol treatments caused a dose-dependent increase in VAT leptin levels in rats. Leptin levels in the low-, middle- and high-dose groups increased in VAT by 114% ($P = 0.059$), 133% ($P = 0.029$), and 321% ($P = 0.000$), respectively, and in sera by 36.3% ($P = 0.047$), 42.4% ($P = 0.022$), and 6.89% ($P = 0.698$), compared to the control group respectively (Figure 1).

Chronic ethanol consumption decreased adiponectin in VAT and sera

Chronic ethanol treatments caused a dose-dependent decrease in adiponectin in both VAT and sera of rats. Adiponectin levels decreased gradually with increasing ethanol levels.

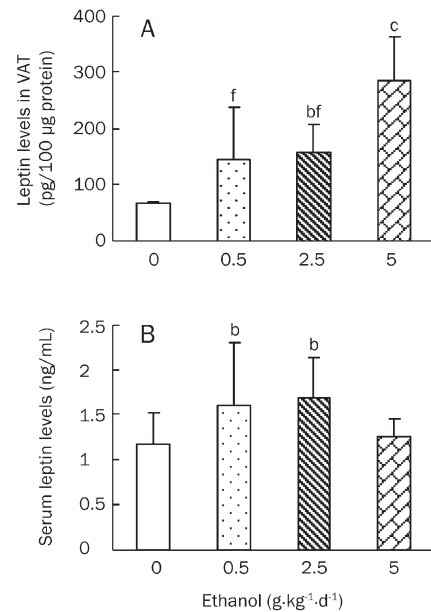


Figure 1. Effects of chronic ethanol treatments on leptin levels in VAT (A) and sera (B) of rats. Wistar rats were fed with edible ethanol at doses of 0, 0.5, 2.5, and 5.0 g·kg⁻¹·d⁻¹ for 22 weeks. VAT was obtained from epididymal and perirenal fat pads and blood samples were collected. Leptin levels in both VAT and sera were measured by ELISA. Values are given as mean \pm SD ($n = 12$ in the 0 and 0.5 g·kg⁻¹·d⁻¹ groups; $n = 11$ in the 2.5 g·kg⁻¹·d⁻¹ group; $n = 10$ in the 5.0 g·kg⁻¹·d⁻¹ group). ^b $P < 0.05$, ^c $P < 0.01$ vs 0 g·kg⁻¹·d⁻¹ group; ^f $P < 0.01$ vs 5 g·kg⁻¹·d⁻¹ group.

Adiponectin levels in low-, middle- and high-dose groups decreased in VAT by 19.37% ($P = 0.310$), 32.76% ($P = 0.095$) and 43.95% ($P = 0.030$), respectively, and in sera by 15.78% ($P = 0.196$), 18.73% ($P = 0.087$), and 39.96% ($P = 0.001$), compared to the control group respectively (Figure 2).

Chronic ethanol treatments increased resistin in VAT and sera

We examined the dose-dependent effects of ethanol on VAT resistin expression. Resistin levels in low-, middle-, and high-dose groups increased in VAT by 1.46% ($P = 0.807$), 10.4% ($P = 0.091$), and 16.6% ($P = 0.014$), respectively, and in sera by 43.5% ($P = 0.011$), 46.4% ($P = 0.001$), and 40.4% ($P = 0.005$), compared to the control group respectively (Figure 3).

Table 1. Characterization of rats fed with different dosages of edible ethanol for 22 weeks. ^b $P < 0.05$, ^c $P < 0.01$ vs ethanol 0 g·kg⁻¹·d⁻¹ group.

Ethanol Intake (g·kg ⁻¹ ·d ⁻¹)	0	0.5	2.5	5.0
Final Number:	12	12	11	10
Initial BW (g)	220.3 \pm 13.1	224 \pm 16.9	222.3 \pm 18.6	224.9 \pm 18.3
Final BW (g)	477.7 \pm 34.5	456.7 \pm 62.6	418.67 \pm 41.89 ^b	393.8 \pm 56.5 ^c
Epididymal fat mass (% of BW)	0.9 \pm 0.3	0.9 \pm 0.2	0.95 \pm 0.2	1.2 \pm 0.3 ^p
Perirenal fat mass (% of BW)	1.28 \pm 0.58	1.09 \pm 0.38	1.13 \pm 0.56	1.19 \pm 0.71
FBG (mmol/L)	3.46 \pm 0.23	3.44 \pm 0.38	3.50 \pm 0.22	3.6 \pm 0.36
FINS (mIU/L)	20.6 \pm 5.2	22.0 \pm 5.6	20.47 \pm 11.67	28.4 \pm 6.1 ^b
HOMA-IR	3.2 \pm 1.2	3.4 \pm 1.0	3.18 \pm 1.1	4.5 \pm 1.0 ^p

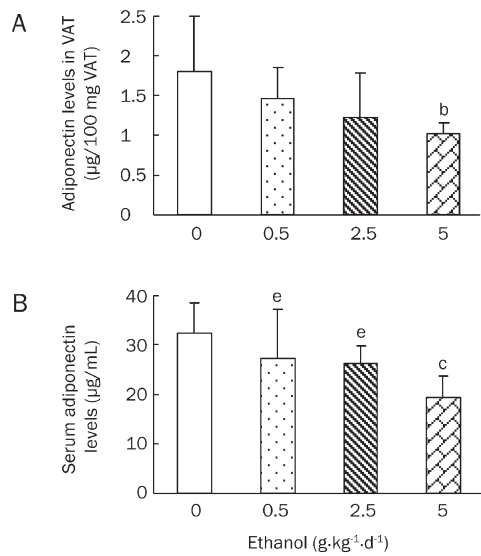


Figure 2. Effects of chronic ethanol treatment on adiponectin levels in VAT (A) and sera (B) of rats. Wistar rats were fed with edible ethanol at doses of 0, 0.5, 2.5, and 5.0 g·kg⁻¹·d⁻¹ for 22 weeks. VAT was obtained from epididymal and perirenal fat pads and blood samples were collected. Adiponectin levels in both VAT and sera were measured by ELISA. Values are given as mean±SD ($n=12$ in the 0 and 0.5 g·kg⁻¹·d⁻¹ groups; $n=11$ in the 2.5 g·kg⁻¹·d⁻¹ group; $n=10$ in the 5.0 g·kg⁻¹·d⁻¹ group). ^b $P<0.05$, ^c $P<0.01$ vs 0 g·kg⁻¹·d⁻¹ group; ^e $P<0.05$ vs 5 g·kg⁻¹·d⁻¹ group.

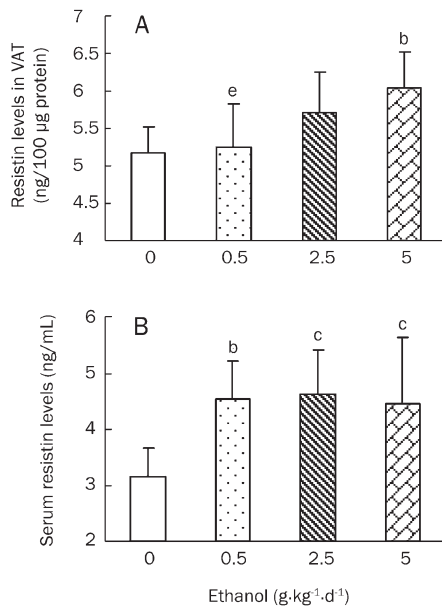


Figure 3. Effects of chronic ethanol treatment on resistin levels in VAT (A) and sera (B) of rats. Wistar rats were fed with edible ethanol at doses of 0, 0.5, 2.5, and 5.0 g·kg⁻¹·d⁻¹ for 22 weeks. VAT was obtained from epididymal and perirenal fat pads and blood samples were collected. Resistin levels in both VAT and sera were measured by ELISA. Values are given as mean±SD ($n=12$ in the 0 and 0.5 g·kg⁻¹·d⁻¹ groups; $n=11$ in the 2.5 g·kg⁻¹·d⁻¹ group; $n=10$ in the 5.0 g·kg⁻¹·d⁻¹ group). ^b $P<0.05$, ^c $P<0.01$ vs 0 g·kg⁻¹·d⁻¹ group; ^e $P<0.05$ vs 5 g·kg⁻¹·d⁻¹ group.

Chronic ethanol treatments raised visfatin in VAT and sera

Chronic ethanol treatments caused a significant dose-dependent increase in serum visfatin levels. Visfatin levels increased gradually with increasing ethanol. Visfatin levels among low-, middle- and high-dose groups increased in VAT by 31.0% ($P=0.101$), 11.9% ($P=0.500$), and 89.2% ($P=0.000$), respectively, and in sera by 33.9% ($P=0.051$), 85.5% ($P=0.000$), and 96.8% ($P=0.000$), in relation to the control group respectively (Figure 4).

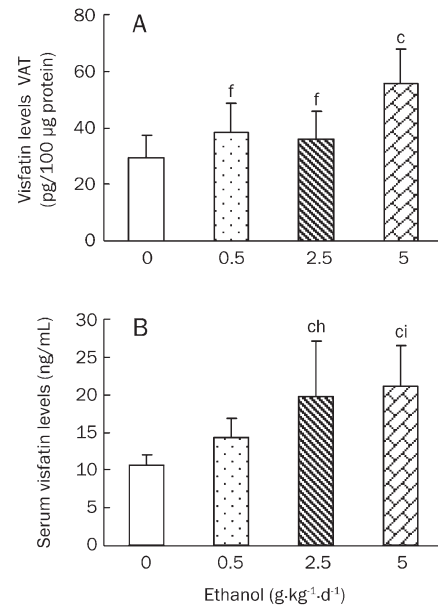


Figure 4. Effects of chronic ethanol treatment on visfatin levels in VAT (A) and sera (B) of rats. Wistar rats were fed with edible ethanol at doses of 0, 0.5, 2.5, and 5.0 g·kg⁻¹·d⁻¹ for 22 weeks. VAT was obtained from epididymal and perirenal fat pads and blood samples were collected. Visfatin levels in both VAT and sera were measured by ELISA. Values are given as mean±SD ($n=12$ in the 0 and 0.5 g·kg⁻¹·d⁻¹ groups; $n=11$ in the 2.5 g·kg⁻¹·d⁻¹ group; $n=10$ in the 5.0 g·kg⁻¹·d⁻¹ group). ^c $P<0.01$ vs 0 g·kg⁻¹·d⁻¹ group; ^f $P<0.01$ vs 5 g·kg⁻¹·d⁻¹ group; ^{ch} $P<0.05$, ^{ci} $P<0.01$ vs 0.5 g·kg⁻¹·d⁻¹ group.

Chronic ethanol treatments decreased cartonectin in VAT

Chronic ethanol treatments caused a dose-dependent decrease in VAT cartonectin contents in rats. Cartonectin levels descended gradually with increasing ethanol. Cartonectin levels in low-, middle- and high-dose groups descended in VAT by 5% ($P=0.679$), 9% ($P=0.455$), 33% ($P=0.009$), compared to the control group respectively. Whereas changes in serum cartonectin levels in rats with or without ethanol were not statistically significant (Figure 5).

Chronic ethanol treatments increased TNF-α in serum

The effect of chronic ethanol exposure on serum TNF-α levels was assessed by ELISA. Enhanced serum TNF-α levels were observed with chronic ethanol exposure. Specifically, low-,

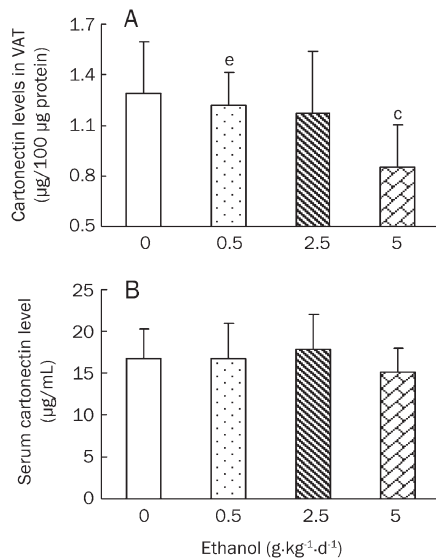


Figure 5. Effects of chronic ethanol treatment on cartonectin levels in VAT (A) and sera (B) of rats. Wistar rats were fed with edible ethanol at doses of 0, 0.5, 2.5, and 5.0 g·kg⁻¹·d⁻¹ for 22 weeks. VAT was obtained from epididymal and perirenal fat pads and blood samples were collected. Cartonectin levels in both VAT and sera were measured by ELISA. Values are given as mean±SD (*n*=12 in the 0 and 0.5 g·kg⁻¹·d⁻¹ groups; *n*=11 in the 2.5 g·kg⁻¹·d⁻¹ group; *n*=10 in the 5.0 g·kg⁻¹·d⁻¹ group). ^c*P*<0.01 vs 0 g·kg⁻¹·d⁻¹ control group; ^a*P*<0.05 vs 5 g·kg⁻¹·d⁻¹ group.

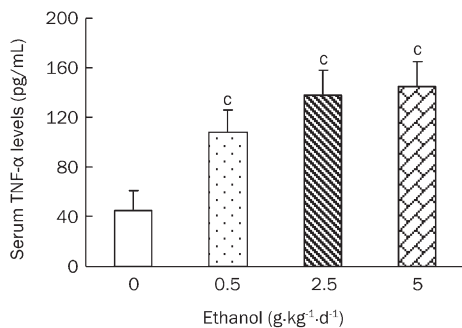


Figure 6. Effects of chronic ethanol treatment on serum TNF-α levels. Wistar rats were fed with edible ethanol at doses of 0, 0.5, 2.5, and 5.0 g·kg⁻¹·d⁻¹ for 22 weeks. Serum TNF-α levels were quantified by ELISA. Values are given as mean±SD (*n*=12 in the 0 and 0.5 g·kg⁻¹·d⁻¹ groups; *n*=11 in the 2.5 g·kg⁻¹·d⁻¹ group; *n*=10 in the 5.0 g·kg⁻¹·d⁻¹ group). ^c*P*<0.01 vs 0 g·kg⁻¹·d⁻¹ group.

middle-, and high-dose groups had increased serum TNF-α levels of 1.2-, 2.1-, and 2.2-fold, respectively, when compared with the control group (Figure 6).

Positive correlation between VAT and serum levels of adiponectin, leptin, resistin, and visfatin

We conducted a correlation analysis between VAT contents and serum levels of all adipokines measured in this study. The following Pearson's coefficient correlations and significance values were calculated: leptin, *r*=0.467, *P*=0.025;

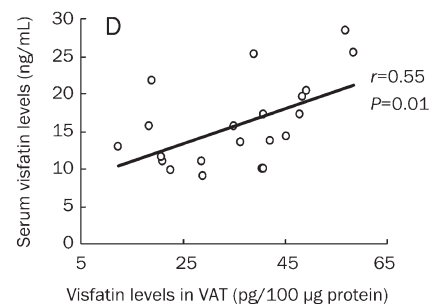
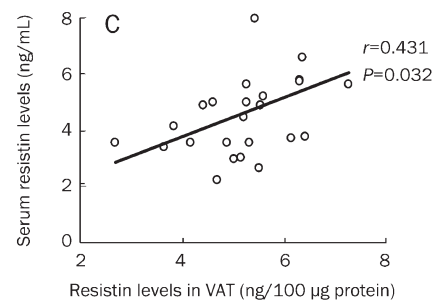
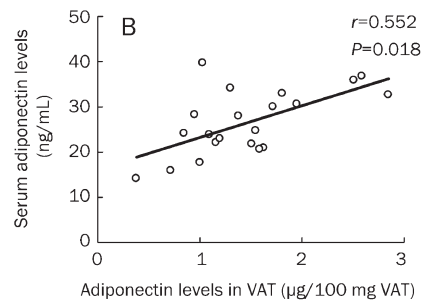
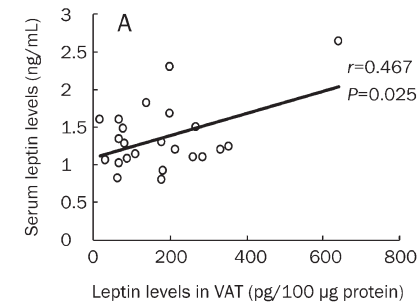


Figure 7. Correlation between VAT and serum adipokine levels of rats. A correlation analysis was applied to test for significant correlations as follows: (A) correlations between VAT and serum leptin levels; (B) correlations between VAT and serum adiponectin levels; (C) correlations between VAT and serum resistin levels; (D) correlations between VAT and serum visfatin levels.

adiponectin, *r*=0.552, *P*=0.018; resistin, *r*=0.431, *P*=0.032; and visfatin, *r*=0.55, *P*=0.01 (Figure 7). No correlation was observed between VAT and serum levels of cartonectin (data not shown).

Discussion

The main findings of this study were that rats treated with

ethanol for 22 weeks had decreased adiponectin and cartonec-tin levels in VAT but increased leptin, resistin and visfatin levels in both VAT and sera. These effects were dose-depend-ent. Positive correlations between VAT and serum levels of leptin, adiponectin, resistin and visfatin were evident to a cer-tain degree.

The exact ethanol dosages that constitute light, moderate and heavy drinking remain controversial. Light, moder-ate and heavy drinkers are generally considered to consume between 1.0–14.9, 10–29.99, and ≥ 30 g·d⁻¹ of ethanol, respec-tively^[16]. Ethanol effects on insulin sensitivity might be dose-dependent so the present study included low-, middle-, and high-dose groups. Ethanol dosages among these three treatment groups were established using a body surface area conversion ratio between rats and humans. Thus, rats were treated with ethanol at 0.5, 2.5, and 5 g·kg⁻¹·d⁻¹ based upon a human (60 kg) equivalent dose conversion equal to 4.8, 24, and 48 g·d⁻¹, respectively. Based on the literature, these three increasing ethanol treatments in rats were equivalent to light, moderate and heavy drinking among humans.

Direct effects of ethanol on adipokines can be observed in cell cultures; however, ethanol effects at the organism level could not be thoroughly demonstrated in cell cultures. In this study, rats were treated with ethanol by gastric tube to ensure consistent ethanol intake. In addition, a 22-week etha-nol treatment was conducted to mimic long-term exposure. The adipokines detected in the present study were derived mainly from adipose tissues and were measured in both adi-pose tissues and sera of rats simultaneously. This approach facilitated an examination of the correlations between adipose tissue and serum levels of adipokines. It also allowed for the investigation of potential drug therapeutic targets for meta-bolic syndrome, such as insulin resistance. Moreover, our use of adipose tissue focused on VAT, which is closely linked to metabolic syndrome^[17]. This focus on VAT helps to address the insufficiencies of past human studies in which only serum adipokine levels were measured but not their tissue of origin because adipose tissue is inconvenient to obtain.

Western blotting is a qualitative and semi-quantitative anal-ysis method to determine adipokine expression in adipose tis-sues. However, Western blotting is not fully quantitative and is difficult to use to measure serum adipokines levels. ELISA is a qualitative and quantitative laboratory method with high sensitivity, powerful specificity and excellent precision. It has been widely used to quantify cytokine levels. So we applied ELISAs in this study to detect variations in VAT adipokine levels among rats receiving different dosages of ethanol.

We found that chronic ethanol ingestion decreased rat BW to different degrees. This is consistent with findings by Oبرا-dovic *et al*^[18] that ethanol-treated animals had reduced diet intake in comparison to water-treated controls when fed *ad libitum*. However, pair-feeding abolished the effects of alcohol on body weight when the supplied food was limited in the animals treated with and without alcohol^[19], suggesting that chronic ethanol intake might interfere with the nutritional status of rats and decrease body weight, thus not directly

affecting body weight^[20]. Although chronic ethanol ingestion resulted in decreased body weight, no significant differences in body weight were observed among low-, middle-, and high-dose ethanol treatments. However, notable differences in adipokine levels were measured across the different treatment groups. Based on these observations, altered adipokine levels were not caused by changes in body weight.

We noticed that the body weights of ethanol-treated rats decreased along with a gradual decline in insulin sensitiv-ity, while the relative epididymal adipose tissue weights increased. Fujioka *et al*^[21] found that increased glucose tol-erance after dietary-induced weight loss were significantly related to VAT decreases, even after adjustments for overall weight loss. Goodpaster *et al*^[22] demonstrated that the per-centage decrease in VAT, rather than that of subcutaneous abdominal adipose tissues and thigh muscle composition, cor-related significantly with changes in insulin sensitivity after weight loss in obese, sedentary women and men. This line of evidence suggests that VAT, rather than body weight, are correlated with adiponectin levels and insulin sensitivity^[23]. These phenomena suggest that accumulation of VAT is closely related to insulin resistance.

In the current study, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were also determined and found to be significantly elevated in all three ethanol exposure groups (data not shown). These data sug-gest ethanol-induced liver injury, a finding that is consistent with our earlier research using an identical drinking model^[12]. Ethanol-induced liver injury may partly contribute to the increased insulin resistance index. Wiest *et al*^[24] demonstrated that leptin, adiponectin, resistin and visfatin did not undergo hepatic clearance. They also observed similar leptin and adi-ponectin levels in patients with and without liver cirrhosis, while systemic visfatin levels were decreased and resistin levels were increased in liver cirrhosis^[24]. Therefore, changes in leptin, adiponectin and visfatin levels in our study were independent of ethanol-induced liver injury. In addition, reduced levels of adiponectin may establish a proinflamma-tory milieu that contributes to liver injury^[25]. The ethanol-induced liver injury may partly contribute to elevated resistin levels, whereas direct effects of ethanol on resistin levels cannot be excluded. It may be possible that both direct and indirect effects of ethanol on resistin concentrations lead to higher serum levels in low- and middle-dose groups than that in VAT. The relationship of adipokine changes to liver injury requires further research. In addition, TNF- α is released from Kupffer cells during chronic alcohol ingestion. Decreased adi-ponectin levels may contribute to the increased TNF- α levels we observed because the actions of TNF- α are opposed by adi-ponectin.

We observed that chronic ethanol treatments decreased adi-ponectin levels and increased resistin contents in both VAT and sera, accompanied with an elevated HOMA-IR index. These effects were dose-dependent. Similarly, decreased circu-lating adiponectin concentrations were observed in Wistar rats or mice chronically exposed to ethanol at 17–19 g·kg⁻¹·d⁻¹ for

4 to 6 weeks^[26, 27]. Pravdová *et al*^[28] demonstrated that alcohol (6% ethanol) intake for 28 d decreased resistin mRNA expression in adipose tissue but significantly increased resistin levels in serum. Elevated serum resistin levels could contribute to reduced size of adipocytes, elevated glycemia, attenuated insulin-stimulated glucose transport in adipocytes, increased FFA and TNF- α and decreased adiponectin levels^[28, 29]. These alterations could contribute to the development of insulin resistance. In contrast, Sierksma *et al*^[30] described that daily consumption of 40 g ethanol increased plasma adiponectin levels and the insulin sensitivity index of healthy, middle-aged male subjects. Differences in species and duration may contribute to the different changes in adiponectin levels. Way *et al*^[31] reported that experimental obesity in rodents is associated with severely defective resistin expression and that resistin levels are negatively correlated with insulin resistance. Serum resistin levels weakly correlated with insulin resistance in Chinese children and adolescents^[32]. These different experimental results suggest that the adiponectin and resistin variation is strongly associated with the study species, treatment duration and dosage and alcoholic beverage category.

To date, no data are available on the effects of ethanol on visfatin and cartonectin levels, although both are adipokines closely related to insulin sensitivity. Therefore, it seems important to provide data on the effects of ethanol on visfatin and cartonectin levels. Visfatin functions dually in an endocrine role modulating insulin sensitivity in peripheral organs; and an autocrine or paracrine role on VAT that facilitates differentiation and fat deposition^[33]. The present study shows that chronic ethanol consumption elevated serum visfatin levels in a dose-dependent fashion. Considering the dual functions of visfatin, these increases might be a compensatory mechanism by which ethanol-exposed rats attempt to prevent the development of insulin resistance, or a marker for insulin resistance in the ethanol intake doses and treatment period of the current study.

We also found that chronic ethanol treatments decreased cartonectin levels in VAT in a dose-dependent fashion, similar to adiponectin decreases in VAT. This observation supports that cartonectin is the adiponectin paralog and a member of C1q/TNF- α molecular superfamily^[5]. Wolfing *et al* have reported that cartonectin could induce adiponectin and resistin secretions from murine adipocytes, *in vitro*, in a dose-dependent manner^[6]. Similarly, we found that chronic ethanol treatments caused a dose-dependent decrease in adiponectin and cartonectin levels in rats but increased resistin levels. The experimental consequences might be mainly relevant to different study systems, *in vivo* and *in vitro*. Taken together, it is not exclusive that adipose tissue-derived cartonectin might exert an autocrine or paracrine function on adipokine secretions from local adipose tissue, potentially modulating insulin resistance in our ethanol-exposed rats.

Our correlation analysis showed a positive correlation between VAT and serum levels of adiponectin, leptin, resistin and visfatin. This positive correlation supports the idea that variations in serum adipokine levels after ethanol treatment

might be due to changes in VAT synthesis of adipokines. Therefore, these adipokines, with the exception of cartonectin, may be good targets for developing drug therapies for diabetes.

Changes in VAT adipokines had similar tendency to changes in sera adipokines in general with increasing ethanol treatments; however, there were notable exceptions. For example, VAT resistin levels did not increase significantly but serum resistin levels did markedly increase in low- and middle-dose groups. This disparity between VAT and serum levels of resistin suggests that chronic ethanol intake enhanced resistin secretion from adipose tissues that might participate in the development of insulin resistance. In the high-dose group, VAT leptin levels increased but serum leptin levels did not significantly change, possibly because high ethanol intake inhibited leptin secretion. The leptin deficiency resulted in the loss of inhibited fat synthesis, which in turn resulted in an accumulation of VAT. Based on these findings, we hypothesize that chronic ethanol intake might cause secretion disorders of adipokines in VAT.

Another observation of the present study was that adipokines presented distinct sensitivity to ethanol. High-dose treatments of ethanol (5 g·kg⁻¹·d⁻¹) for 22 weeks depressed adiponectin and cartonectin levels in the VAT of rats by 43.95% and 33%, respectively. This high-dose group also showed elevated leptin, resistin and visfatin levels in the VAT of rats by 321%, 16.6%, and 89.2%, respectively. In rat serum, ethanol decreased adiponectin levels by 39.96% and increased leptin, resistin and visfatin levels by 6.89%, 40.4%, and 96.8%, respectively, but did not affect cartonectin levels. In VAT, leptin variations were the highest, followed by visfatin changes, whereas in serum, visfatin alterations were the highest and leptin changes were the lowest (Figure 8). Relatively, visfatin variations in VAT and serum were the most substantial of the adipokines studied, indicating that visfatin is highly sensitive to ethanol. Based on this observation, we propose that visfa-

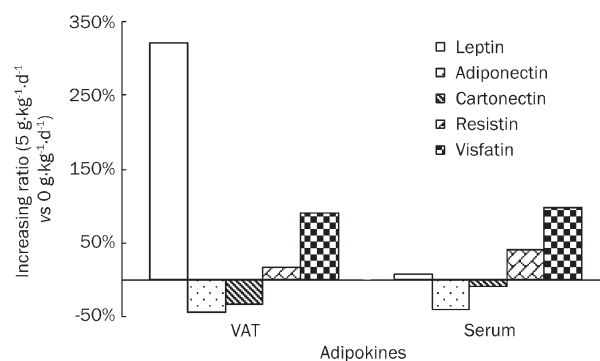


Figure 8. Comparison of the increasing ratio of respective adipokine levels in both VAT and serum of rats. Wistar rats were fed with edible ethanol at 5.0 g·kg⁻¹·d⁻¹ for 22 weeks. VAT was obtained from epididymal and perirenal fat pads and blood samples were collected. Adipokine levels in both VAT and serum were measured by ELISA. The increasing ratio of adipokine levels (vs 0 g·kg⁻¹·d⁻¹ group) in both VAT and serum of rats is indicated in the bar graph.

tin might change earlier than other adipokines as a result of ethanol-induced insulin resistance. Thus, visfatin alterations might be a promising biomarker for prevention and diagnosis for metabolic syndrome, such as VAT accumulation and insulin resistance. Araki *et al*^[34] reported that plasma visfatin levels were significantly higher in obese children than in non-obese, control children. They suggest that plasma visfatin levels were a specific biomarker for VAT accumulation in these obese children.

In conclusion, chronic ethanol consumption affected adipokine levels in both VAT and sera in a dose-dependent manner, with the exception of serum cartonectin. These altered adipokine levels may participate in chronic ethanol-induced insulin resistance and may be targets for developing drug therapies for diabetes. Visfatin is a promising biomarker for the prevention and diagnosis of metabolic syndrome, such as insulin resistance. However, the precise underlying mechanisms and potential significance of chronic ethanol consumption on changes in adiponectin, leptin, resistin, visfatin and cartonectin levels require further investigation.

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Author contribution

Ling GAO and Jia-jun ZHAO designed the research; Heng-cai YU, Li FENG and Ming-feng CAO performed the research; Heng-cai YU and Xiu-yun JIANG analyzed the data; Ling GAO, Heng-cai YU and Si-ying LI prepared the manuscript.

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