

Comparison of Gene Transcription between Subcutaneous and Visceral Adipose Tissue in Chinese Adults

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Abstract. Obese individuals with fat stored in visceral adipose tissue (VAT) generally suffer greater adverse metabolic consequences than those with fat stored predominantly in subcutaneous adipose tissue (SAT), but its molecular basis is not completely understood. We isolated paired samples of SAT and VAT from 15 lean and 15 obese subjects and systematically compared the transcription level of genes that may determine fat distribution and metabolic sequelae between SAT and VAT using quantitative real-time PCR. We found that, leptin levels were lower in VAT than SAT, for both lean and obese subjects. In lean subjects, tumor necrosis factor- α (TNF- α) was expressed equally in both fat depots, while toll-like receptor 4 (TLR4) and glucocorticoid receptor (GR) showed significantly lower expression in VAT than SAT. In obese subjects, TNF- α and TLR4 expression were significantly higher in VAT than SAT, yet GR expression did not differ in these areas. For all subjects, VAT 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) level was significantly correlated with BMI. GR expression level was significantly correlated with TLR4 expression level. Cultured adipocytes showed higher TLR4 mRNA level after differentiation, and higher TNF- α level after treatment with free fatty acids. These results suggest that there are depot-specific differences in leptin, TNF- α , TLR4 and GR transcriptions in humans. TLR4 signaling and higher 11 β -HSD1 and GR levels in VAT may contribute predominantly to inflammatory factor production and subsequent metabolic sequelae in obese human.

Key words: Subcutaneous adipose tissue, Visceral adipose tissue, TLR4, TNF- α , Inflammation

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OBESITY has become a worldwide epidemic disease due to unhealthy diet and physical inactivity. Prospective studies have shown that excess body fat in the abdomen correlates with increased mortality and risk of glucose intolerance, hyperinsulinemia, hypertriglyceridemia and other features of metabolic syndrome [1, 2]. Abdominal obesity is also a significant risk factor for cardiovascular disease [3]. Previous works showed that surgical removal of visceral fat in mice prevented insulin resistance and glucose intolerance [4, 5]. Visceral adipose tissue (VAT) is morphologically and functionally different from subcutaneous adipose tissue (SAT) [2]. One major difference between these

two fat depots is that free fatty acid (FFA) levels and lipolysis are higher in visceral fat cells than in subcutaneous fat cells. Impaired FFA metabolism may contribute to the insulin-resistant state observed in individuals with visceral obesity [6].

The adipose tissues secrete a variety of adipokines including leptin, adiponectin, monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor (TNF- α). The adipose tissues also express various receptors such as toll like receptor 4 (TLR4), glucocorticoid receptor (GR) and peroxisome proliferator-activated receptor- γ (PPAR- γ). 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) is an enzyme highly expressed in adipose tissues [7, 8]. Most of these genes are elevated in the obese state, with the exception of adiponectin, which exhibits insulin-sensitizing effects [8]. There is accumulating evidence for regional differences in the expression of these genes in VAT versus SAT [9-14]. However, there has been some controversy over the data about regional expression of these genes,

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with differences being seen in some studies but not in other studies [10, 15, 16]. Further more, there is no such study systematically compares the depot-specific transcription levels of these genes between lean and obese groups.

In addition, obesity can be defined as a chronic inflammation state. The infiltrated macrophages and adipocytes themselves can secrete numerous inflammatory cytokines, such as TNF- α , IL-1, IL-6, and MCP-1, which then lead to impaired insulin response in adipocytes [17-22]. TLR4 mediates expression of various inflammatory cytokines and involved in inflammation [23, 24]. TLR4 is reported abundantly expressed in mouse adipose tissue and more recently in human SAT [25, 26]. However, a comparison of TLR4 expression between SAT and VAT is still lacking.

In this study, we systematically compared gene transcription profiles in human VAT and SAT samples from lean and obese groups, aimed to seek differences between VAT and SAT. We found that there are indeed depot-specific differences in leptin, TNF- α , TLR4 and GR transcriptions. These regional differences in gene transcription may help to define functional differences between SAT and VAT, and associated metabolic sequelae in humans.

Materials and Methods

Subjects

All patients were Chinese and from the Department of Surgery, the First Hospital Affiliated to Nanjing Medical University. The study population contained 20 lean subjects (11 women and 9 men), with a mean age of 44.6 ± 14.7 years, and a BMI of $22.7 \pm 1.8 \text{ kg/m}^2$, and 15 obese subjects (6 women and 9 men), with a mean age of 54.3 ± 10.3 years, and a BMI of $28.2 \pm 1.2 \text{ kg/m}^2$. As recommended by the Working Group on Obesity in China (WGOC), we used a BMI of 25 kg/m^2 as the definition for overweight, and a BMI of 28 kg/m^2 as a definition for obesity. Tissue samples were obtained from patients undergoing lower abdominal surgery under general anesthesia. Patients were fasted at least 8 h, not treated with any hormones that may influence metabolism prior to the acquisition of samples. None of the patients had diabetes and none were on medications known to influence adipose mass or metabolism. VAT samples were isolated from

greater omentum and SAT samples were depots below the skin in the abdominal region. The study protocol was approved by the research ethics committee of Nanjing Medical University.

Metabolic measurements

Blood samples were collected into a BD Vacutainer[®] tube from subjects after 12 h overnight fast. Levels of fasting blood glucose were determined by glucose oxidase- peroxidase, peroxidase anti-peroxidase (GOD-PAP) method (Kehua Bioengineering, Shanghai, China). Cholesterol levels were determined by cholesterol oxidase-peroxidase, 4-aminophenazone (CHOD-PAP) method (HUMAN, Taunusstein, Germany), and triglyceride levels were determined by glycerol phosphate oxidase-p-aminophenazone (GPO-PAP) method (Daiichi Chemical, Tokyo, Japan). All blood analysis procedures were performed according to the kit manufacturer instructions.

Tissue samples

To assess depot-specific gene expression, paired samples of SAT and VAT were isolated from 15 lean and 15 obese subjects (Table 1), immediately frozen in liquid nitrogen, and then saved in -80°C for total RNA extraction and quantitative real-time PCR. For primary cell culture, VAT samples were isolated from another 5 lean subjects with a mean age of 29.2 ± 11.2 years, and a BMI of $22.5 \pm 2.3 \text{ kg/m}^2$. Tissues samples were placed in PBS (Invitrogen, Carlsbad, CA, USA) containing 20 g/L BSA (Sunshine Biotech, Nanjing, China) during the surgery, then immediately transferred to the laboratory.

Primary cell culture

Preadipocytes (stromal-vascular cells) were isolated from VAT samples of five lean subjects. Preadipocytes were cultured as previously described [27]. Briefly, tissue samples were treated with 2 mg/mL collagenase I (Invitrogen) and centrifuged to separate preadipocytes from adipocytes. The supernatant was discarded and preadipocytes were resuspended in growth medium (DMEM/Ham's F-12 medium with 10% Fetal Bovine Serum; Life Technologies, Gaithersburg, MD, USA) and allowed to adhere to the culture dish overnight. The next day, cells were washed and set to cul-

Table 1. Physical and metabolic variables of patients whose samples were used in RT-PCR analysis

Variable	Lean		Obese	
Sex	female (n=9)	male (n=6)	female (n=6)	male (n=9)
Age (years)	49.2±8.7	45.1±18.8	49.8±7.2	56.0±10.6
BMI (kg/m ²)	22.5±1.4	23.1±1.7	28.8±0.9**	28.2±1.4**
Waist circumference (cm)	85.2±4.3	88.2±5.0	96.3±6.1**	95.5±6.1*
Hip circumference (cm)	93.4±3.7	93.8±2.9	100.1±2.0**	99.0±4.2*
Waist-to-hip ratio	0.91±0.04	0.94±0.03	0.96±0.05**	0.97±0.03*
Systolic blood pressure (mmHg)	115.9±15.7	127.2±12.2	126.2±8.7	130.2±18.1
Diastolic blood pressure (mmHg)	71.0±12.4	78.0±9.3	80.8±8.6	86.4±6.0
Cholesterol (mg/L)	4.09±1.03	4.21±0.35	4.18±0.55	4.89±0.94
Triglycerides (mg/L)	1.11±0.81	1.03±0.29	1.31±0.41	1.70±0.90
Plasma glucose (mmol/L)	4.63±0.42	5.03±0.81	6.58±1.75 *	5.54±0.77

Values are means ± SD.

Significant differences between lean and obese groups are indicated by: * $p < 0.05$, and ** $p < 0.01$.

Table 2. Primer sequences for real-time PCR

Gene	Forward and reverse primer (5'-3')
β-Actin	F-ATTGGCAATGAGCGGTTCGCG R-CTCCTGCTTGCTGATCCACATC
Leptin	F-CACCAAAACCCTCATCAAGACA R-CTTTCTGTTTGGAGGAGACTGACT
Adiponectin	F-CGTGATGGCAGAGATGGC R-CCGGTTTCACCGATGTCT
TNF-α	F-CCCCAGGGACCTCTCTAAT R-GGTTTGCTACAACATGGGCTAC
TLR4	F-ATGAGGACTGGTAAGGA R-AGATGATACCAGCACGAC
GR	F-TCTGCGTCTTACCCTCA R-CCAGGTCATTCCCATCA
11β-HSD1	F-GCAAAGGGATCGGAAGAGAGATGG R-GTCATGTCTTCCATGGTGCCAGC

Forward (F) and reverse (R) primers were given for each gene at 5' to 3' direction as specified.

ture until confluent.

For differentiation experiments, cells were allowed to adhere overnight in culture medium, washed and grown until confluence in growth medium (day 0). Thereafter, cells were grown in a differentiation medium (serum-free DMEM/Ham's F-12 medium containing 15 mmol/L NaHCO₃, 15 mmol/L Hepes, 33 mmol/L biotin, 17 mmol/L pantothenate, 66 nmol/L human insulin, 0.2 nmol/L triiodothyronine, 1 μmol/L dexamethasone) (Sigma, St. Louis, MO, USA). To induce maximal differentiation, confluent preadipocytes were supplemented with differentiation medium contain-

ing 0.5 mmol/L 3-isobutyl-1-methylxanthine (Sigma) during the first 3 days. After 14 to 16 days of differentiation culture, most preadipocytes were induced into mature adipocytes. To examine the role of FFA in adipocytes, differentiated adipocytes were exposed to 500 μg/mL FFA (Sigma) in serum-free DMEM/Ham's F-12 medium for 24 h.

RNA preparation and quantitative real-time PCR

Total RNA was extracted from fat tissues or cultured adipocytes using TRIzol (Invitrogen) and quantified with real-time PCR. Two micrograms of total RNA was reverse-transcribed with 200 U Moloney murine leukemia virus reverse transcriptase (M-MuLV, Promega, Madison, WI, USA), and in the presence of 0.5 mmol/L deoxynucleotide triphosphate, 25 U RNase inhibitor, and 0.5 μg N9 random primers, in a 25-μL volume. PCR primers (Table 2) were designed by Primer5 software. Each quantitative real-time PCR was carried out in triplicate, in a 25-μL volume of SYBR Green Real-time PCR Master Mix (Toyobo, Osaka, Japan). The PCR program was as follows: 10 s at 95°C, followed by 40 cycles of 30 s at 94°C, 30 s at 60°C, 40 s at 72°C, and 5 s at 80°C on the plate reader (Rotor Gene-3000, Corbett Research, Sydney, Australia).

The mean value of triplicates for each sample was calculated, and expressed as cycle threshold (Ct). For each gene studied, the amount of gene expression was then calculated as ΔCt, the difference between the Ct

value of the sample and the Ct value of β -actin, which used as an endogenous control. The relative expression level was evaluated by comparative delta-delta Ct method, and the software tool, *REST-384*[®] [28]. Expression for each gene in lean SAT is arbitrarily set at 1 to facilitate comparison between SAT and VAT and comparison between lean and obese subjects.

Statistical analysis

Statistical analysis was performed with SPSS 11.0. Non-normally distributed parameters were logarithmically transformed to approximate a normal distribution. Expression levels were compared in paired samples of SAT and VAT from both lean and obese subjects, by expressing results as a ratio to lean SAT expression. A paired Student's t-test was used to compare gene expression between SAT and VAT. An unpaired t-test was used to compare gene expression between lean and obese subjects. Linear relationships were assessed by least squares regression analysis.

Results

Fat depot-specific gene expression

The physical and metabolic characteristics of lean and obese subjects are presented in Table 1. As expected, most of the characteristics associated with metabolic syndromes were significantly higher among those who were obese. Previous study determined that waist circumference and waist-to-hip ratio (WHR) showed good correlation coefficients with visceral fat determined by computed tomography (CT) [29]. In both female and male groups, the waist circumference and WHR of obese were significantly larger than that of lean subjects (Table 1), suggesting there were more visceral fats in obese subjects.

The mRNA levels of important genes expressed by adipocytes were statistically compared between lean and obese groups. These genes were leptin, adiponectin, TNF- α , TLR4, GR, and 11 β -HSD1 (Fig. 1). In obese subjects, adiponectin mRNA level was significantly smaller in VAT samples than SAT samples. In addition, SAT samples from obese subjects showed significantly lower expression of TNF- α , TLR4 and GR, compared to lean subject SAT samples. In contrast, 11 β -HSD1 expression was significantly higher in

obese subject SAT than that of lean subjects. Overall, expression levels of TNF- α , TLR4, GR and 11 β -HSD1 were significantly elevated in the VAT of obese patients, compared to lean patients.

For both patient groups, leptin mRNA expression level was significantly higher in the SAT than VAT (lean group VAT/SAT ratio, 0.44 ± 0.15 , $p < 0.01$, obese group VAT/SAT ratio, 0.48 ± 0.24 , $p < 0.05$, Fig. 1A). Adiponectin mRNA expression showed no difference in SAT and VAT expression levels, for either lean or obese subjects (Fig. 1B). In lean subjects, TNF- α was expressed equally in SAT and VAT. However, in obese subjects, TNF- α expression was 2.73-fold higher in VAT than SAT (Fig. 1C).

TLR4 mRNA was expressed in human adipose tissues. As shown in Fig. 1D, TLR4 expression level was lower in VAT than SAT in lean subjects (VAT/SAT ratio, 0.64 ± 0.15 , $p < 0.01$). However, in obese subjects TLR4 expression level was higher in VAT (VAT/SAT ratio, 1.77 ± 0.64 , $p < 0.01$).

GR mRNA expression was higher in SAT than VAT in lean subjects (VAT/SAT ratio: 0.77 ± 0.13 , $p < 0.05$), but expressed equally in obese subjects (Fig. 1E). No differences were found between SAT and VAT expression of 11 β -HSD1 mRNA, in either lean or obese subjects (Fig. 1F).

Gene expression levels of SAT and VAT in female subject were also analyzed. In lean female subjects, TLR4 mRNA expression level was significantly higher in SAT than VAT ($p < 0.05$). In obese female subjects, TNF- α mRNA showed significantly lower expression in SAT than VAT ($p < 0.05$). In addition, gender differences of gene expression were not found in mRNA levels of TNF- α , TLR4, GR and 11 β -HSD1 in neither SAT nor VAT.

Correlation analysis across all patients

The relationship between mRNA levels and BMI in all patients revealed that 11 β -HSD1 levels in SAT and VAT were both significantly correlated with BMI (SAT $r^2 = 0.262$, $p = 0.0038$; VAT $r^2 = 0.1826$, $p = 0.0185$; Fig. 2 A, B). Furthermore, GR mRNA levels were significantly correlated with TLR4 levels in both SAT ($r^2 = 0.6285$, $p < 0.0005$, Fig. 2C) and VAT ($r^2 = 0.2532$, $p = 0.0054$, Fig. 2D).

The relationship between TNF- α and TLR4 mRNA levels was examined for all subjects. There was a positive correlation between TNF- α and TLR4 mRNA

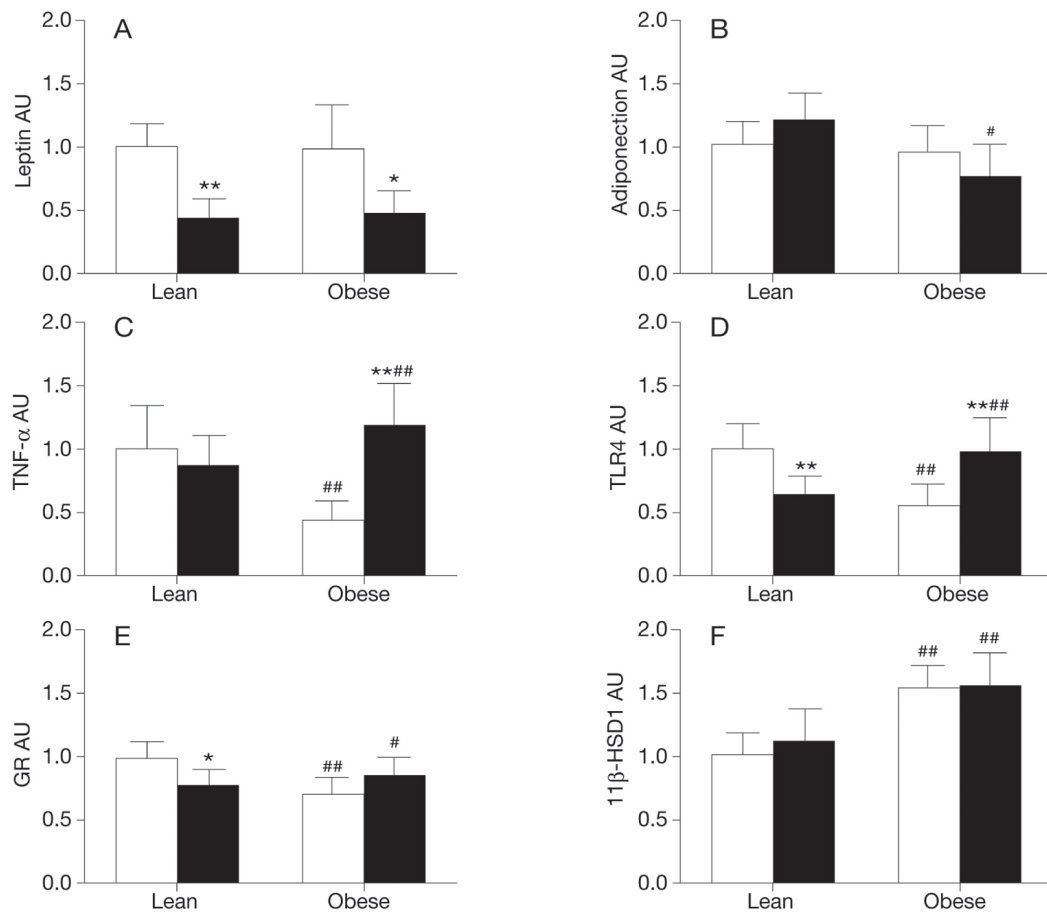


Fig. 1. Gene transcription levels in SAT and VAT samples of lean and obese groups: (A) leptin, (B) adiponectin, (C) TNF- α , (D) TLR4, (E), GR, and (F) 11 β -HSD. White bars = SAT, black bars = VAT. Expression for each gene in lean SAT is arbitrarily set at 1. AU, arbitrary units. Bar height represents mean \pm SEM, $n = 14-15$. Significant difference between SAT and VAT are indicated by: * $p < 0.05$ and ** $p < 0.01$. Significant difference between lean and obese is indicated by: # $p < 0.05$ and ## $p < 0.01$.

level in VAT ($r^2 = 0.5851$, $p < 0.0001$; Fig. 3A). To determine the role of inflammation in obesity, the relationship between TNF- α and patient metabolic characters was examined. TNF- α mRNA level in VATs correlated significantly with BMI ($r^2 = 0.1397$, $p = 0.0419$) and plasma glucose ($r^2 = 0.2547$, $p = 0.0045$, Fig. 3 B, C). However TNF- α mRNA in SATs was not correlated with these characteristics (data not shown). We also found a significant correlation between TLR4 mRNA level in VATs and plasma glucose ($r^2 = 0.1974$, $p = 0.0139$, Fig. 3D).

TLR4 and TNF- α mRNA expression in cultured adipocytes

During the culture of preadipocytes in differentiation medium, the cells gradually adhered, grew to conflu-

ence and became mature adipocytes. An accumulation of lipid droplets could be seen in the adipocyte cytoplasm, indicating total differentiation of preadipocytes.

As shown in Fig. 4A, preadipocytes (day 0) expressed TLR4 mRNA. At the end stage of differentiation, cultured cells expressed higher TLR4 mRNA compared with undifferentiated preadipocytes (1.64-fold, $p < 0.01$).

We examined whether FFA in the culture medium could induce inflammatory signaling in adipocytes. Treatment of differentiated adipocytes with 500 $\mu\text{g/mL}$ FFA for 24 h significantly stimulated TNF- α mRNA expression. Compared with FFA untreated adipocytes, FFA treated adipocytes express higher TNF- α mRNA level (2.67-fold, $p < 0.01$, Fig. 4B).

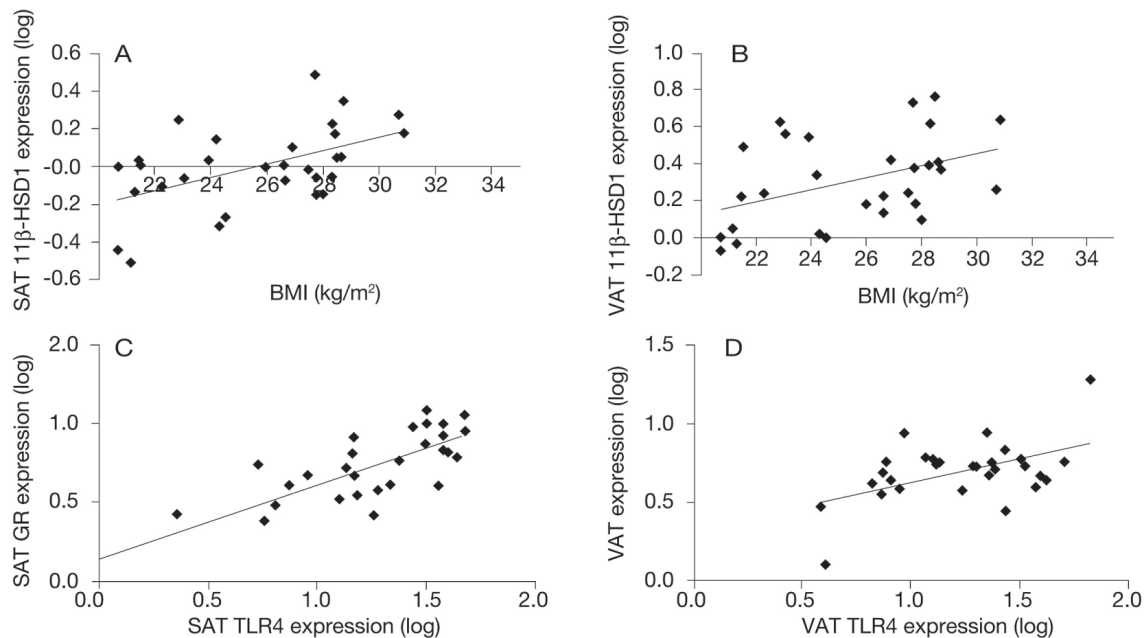


Fig. 2. Scattergrams showing data values and correlation analysis of gene expression levels in SAT and VAT samples. Plot (A), correlation of SAT 11 β -HSD1 level and BMI ($r^2 = 0.262$, $p = 0.0038$). Plot (B), correlation of VAT 11 β -HSD1 level and BMI ($r^2 = 0.1826$, $p = 0.0185$). Plot (C) correlation of SAT GR mRNA level and SAT TLR4 mRNA level ($r^2 = 0.6285$, $p < 0.0005$). Plot (D) correlation of VAT GR mRNA level and VAT TLR4 mRNA level ($r^2 = 0.2532$, $p = 0.0054$). Prior to analysis, data were logarithmically transformed to achieve normal distribution. Twenty nine subjects were involved in this analysis.

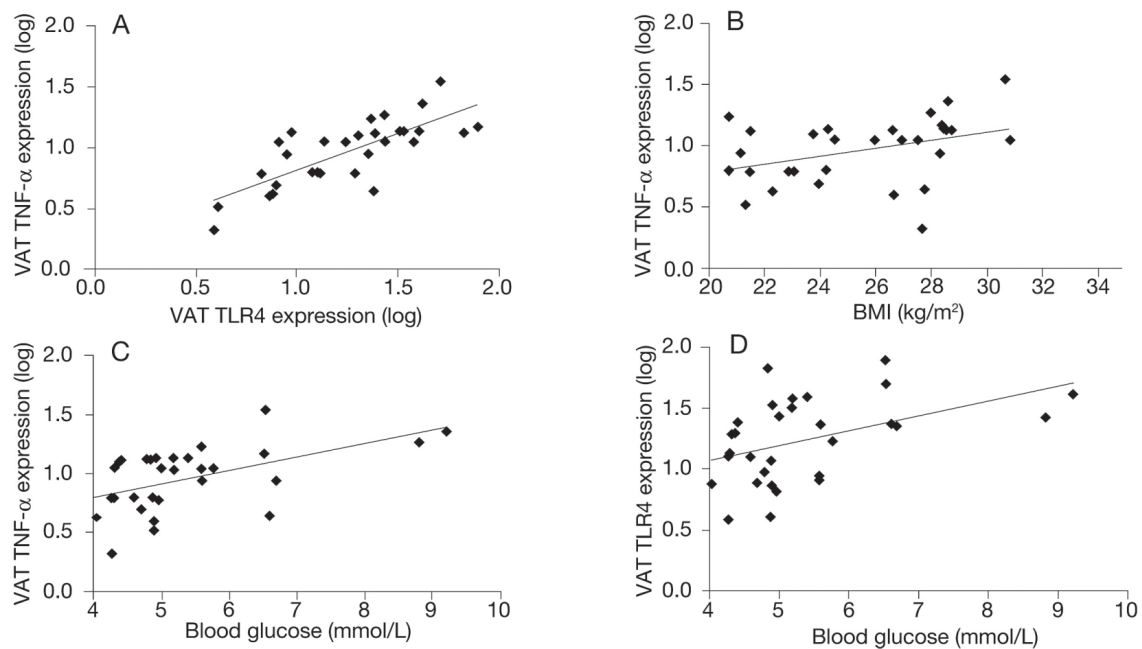


Fig. 3. Scattergrams showing data values and correlation analysis of VAT gene expression levels with selected patient characteristics. VAT TLR4 mRNA levels were correlated with VAT (A) TNF- α mRNA levels ($r^2 = 0.5851$, $p < 0.0001$), (B) BMI ($r^2 = 0.1397$, $p = 0.0419$) and (C) plasma glucose ($r^2 = 0.2547$, $p = 0.0045$). (D) VAT TLR4 mRNA levels also correlate with plasma glucose ($r^2 = 0.1974$, $p = 0.0139$). Data are logarithmically transformed to achieve normal distribution. Thirty subjects were involved in this analysis.

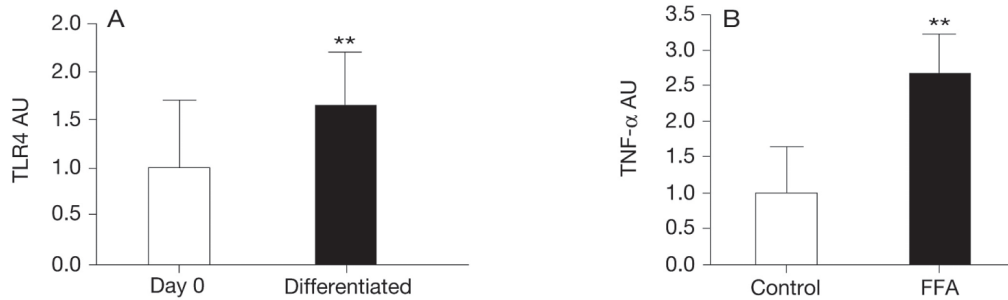


Fig. 4. TLR4 and TNF- α expression in cultured adipocytes. (A) TLR4 mRNA expression is significantly greater in differentiated adipocytes (black bar), than undifferentiated adipocytes (white bar). TLR4 expression level of preadipocytes on day 0 is arbitrarily set at 1. (B) TNF- α expression is significantly higher in mature adipocytes treated with FFA than untreated adipocytes. Differentiated cells not treated with FFA are used as a control, with its TNF- α expression level arbitrarily set at 1. AU, arbitrary units. Data represent five samples for each group, ** $p < 0.01$.

Discussion

In this study, we found that leptin mRNA was highly expressed in the SAT of both lean and obese Chinese patients, which is consistent with findings in obese Caucasians [30, 31]. Adiponectin mRNA expression showed no difference in SAT and VAT expression levels, for neither lean nor obese subjects. Our results are consistent with findings of Yang *et al.*, who also examined Chinese subjects. In contrast, Lihn *et al.* and Desbriere *et al.* found relatively greater adiponectin mRNA levels in SAT than VAT, in a patient group of Caucasian women [9, 10]. Thus, the depot-related expression of adiponectin levels found by Lihn *et al.* and Desbriere *et al.* may be attributable to an ethnic background of the subject group. Interestingly, adiponectin mRNA levels were much lower in the VAT of obese subjects compared with lean subjects. This result suggests there is a depot-specific down-regulation of adiponectin in obesity.

GR is an important nuclear receptor for adipogenesis and metabolism. We found that GR expression level was higher in SAT than VAT in lean subjects, but there was no depot-specific expression in obese subjects. Compared with lean subjects, obese subjects showed higher GR expression in VAT. Our result is not completely consistent with the study from Bronnegard *et al.*, who compared GR expression in men and women, and found that GR is more prevalent in the visceral pre-adipocytes of women, but in men it is more prevalent in subcutaneous pre-adipocytes [13]. One major difference between our study and that of Bronnegard *et al.*, is that they examined pre-adipocytes, whose gene expression may differ from mature adipose tissue.

For both obese and lean patients, there was no fat depot expression difference in 11 β -HSD1. This result is consistent with Desbriere *et al.*, who examined 10 lean and 12 obese Caucasian women [10]. However, Bujalska *et al.* measured 11 β -HSD1 in cultured pre-adipocytes from 16 human adipose samples of SAT and VAT, and did record differences in expression levels [14]. It is possible that the immature *in vitro* preparation is different enough from the mature samples of adipose tissue used in our study. We also found that 11 β -HSD1 mRNA was greater in VAT of obese subjects. Mechanistically, 11 β -HSD1 converts inactive cortisone to active cortisol in adipose tissue, and amplifies intracellular effects of glucocorticoids [32]. It is possible that increased 11 β -HSD1 expression amplifies cortisol action, and subsequently increases differentiation of preadipocytes to mature adipocytes, and a change in the adipokine profile.

Obesity is now considered to be a state of impaired secretory function. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance [22]. Thus, we examined the expression levels of inflammatory factor TNF- α to address a possible role for inflammation in the metabolic syndrome. We found that, in obese subjects, TNF- α mRNA is more highly expressed in VAT than in SAT. Thus, VAT might be a major source of TNF- α , which could help explain visceral-linked insulin resistance in obese individuals. In VAT, TNF- α mRNA showed a significant correlation with BMI and plasma glucose. The lack of correlation of these variables with TNF- α mRNA in SAT suggests that VAT is more closely related to inflammation and metabolic sequelae. In obesity, there is evidence of increased macrophage infiltration in ad-

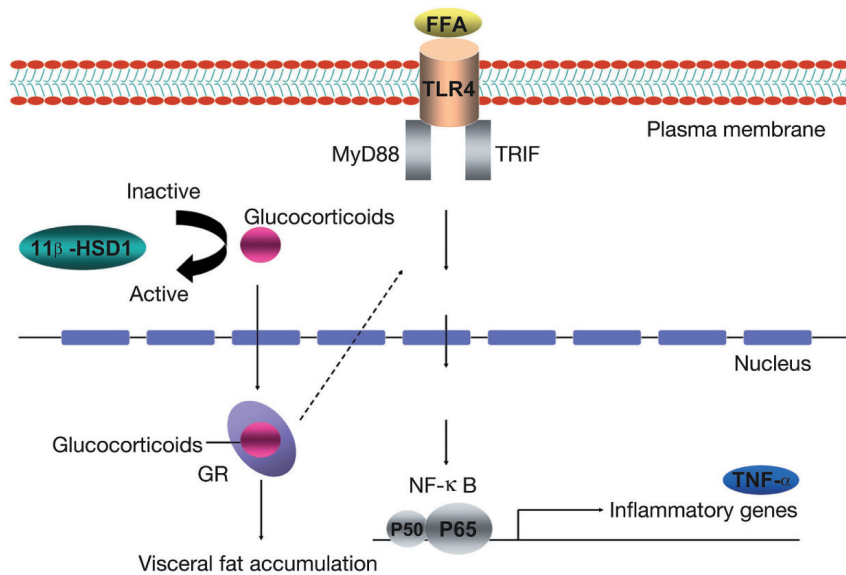


Fig. 5. Schematic overview of pathways mediating inflammation and visceral fat accumulation in obese VAT. Recognition of FFA by TLR4 initiates downstream intracellular signaling transduction through MyD88-dependent or TRIF-dependent pathway. Eventually, the intracellular signaling leads to the activation of nuclear factor κ B (NF- κ B) and the transcription of inflammatory genes [35]. 11 β -HSD1 converts inactive glucocorticoids into active glucocorticoids. Glucocorticoids bind to glucocorticoid receptor (GR), thereby causes visceral fat accumulation. Nuclear receptor GR might regulate the expression of inflammatory genes through the TLR4 pathway. MyD88: Myeloid differentiation primary-response gene 88, TRIF: Toll/interleukin receptor-domain-containing adaptor protein inducing interferon- β .

ipose tissue, where they may be major producers of TNF- α and many other inflammatory mediators [18]. We assessed the infiltration of both SAT and VAT with CD68 bearing macrophages in a small number of lean and obese subjects (data not shown). We demonstrated greater infiltration of macrophages in obese over lean adipose tissue and that VAT had more macrophage infiltration, which are consistent with studies from mouse adipose tissue [18, 33].

TLR4 is an essential component of inflammation and mediates expression of various inflammatory cytokines [23, 24]. Previous studies showed that mouse adipocytes express TLR4, and that lipopolysaccharides and FFA could stimulate expression of inflammatory factors through binding to TLR4 [34]. Song *et al.* found that the mRNA level of TLR4 was remarkably enhanced in fat tissues of *db/db* obese mice [25]. Mice lacking TLR4 were partially protected against high fat diet-induced insulin resistance [34]. A recent study had also showed that human SAT also expressed TLR4 [26]. To our knowledge, our study is the first to compare human TLR4 expression levels in paired samples of SAT and VAT. We found that, in lean subjects, TLR4 mRNA levels were lower in VAT than SAT, while in obese pa-

tients, VAT showed higher TLR4 expression. We also demonstrated that the expression level of TLR4 in VAT showed a significant correlation with TNF- α expression level. In addition, there was a significant correlation between VAT TLR4 mRNA level and plasma glucose. These findings are at least partly compatible with the hypothesis that obesity is associated with increased visceral TLR4 expression.

Both of the adipocytes and macrophages could express TNF- α , TLR4, GR and 11 β -HSD1. Since our sample isolation method could not separate adipose tissue from macrophages, these molecules might be derived from both types of cells. Therefore, we used primary cultures of preadipocytes from VAT to investigate whether visceral TLR4 expression is up-regulated in response to obesity. We examined the expression profile of TLR4 mRNA during adipogenesis. In primary cultured cells, we observed increased TLR4 expression level at the end stage of differentiation. We then investigated whether activation of TLR4 induces TNF- α in human adipocytes. We found that FFA, which is heavily secreted in obese individuals, significantly increased TNF- α mRNA levels in differentiated human adipocytes. Since TLR4 signaling can

be activated by FFA in adipocytes and TLR4 is predominantly expressed in VAT of obese subjects, these data suggest a close link between TLR4 signaling and inflammatory responses in obese visceral fat depots. This subject is ripe for future study.

Interestingly, we found that GR mRNA level was significantly correlated with TLR4 mRNA level, in both SAT and VAT. This result suggests a potential relationship between GR and TLR4. Recently, Ogawa *et al.* found that there was a close link between GR and TLR4. In macrophages, GR could regulate the expression of inflammatory factors through the TLR pathway [35]. Further investigation is necessary to outline to specific relationship of GR to the TLR4 pathway in the adipose tissue.

We regret that we could only measure mRNA levels for our genes of interest. The protein levels of these genes were not examined because human samples were not easily available. Furthermore, we were unable to include mechanistic experimentation aimed to elucidate intrinsic molecular mechanisms of gene interaction. A cell culture system and a siRNA knock-down strategy may be appropriate for follow-up on

our discoveries with TLR4.

In summary, after carefully compared the relative gene transcription levels in paired fat depots, we found that there were indeed differently-expressed leptin, TNF- α , TLR4 and GR in VAT and SAT from lean and obese subjects. Enhanced TLR4 signaling and elevated 11 β -HSD1 and GR levels in VAT might be contributable to inflammation, visceral fat accumulation, and other characteristics of metabolic syndromes related to obesity (Fig. 5). Our study revealed differences between SAT and VAT in molecular level. This may partly explain why visceral obesity is more closely associated with adverse metabolic consequences

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