

Elevated CTSL Gene Expression Correlated with Proinflammatory Cytokines in Omental Adipose Tissue of Patients with Obesity

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Purpose: Cathepsin L (CTSL) and B (CTSB) were lysosomal proteases, and their expression and activity contribute to the progression of inflammation in obese rodents. Our aim was to investigate CTSB and CTSL expression in omental adipose tissue (AT) of patients with obesity and to correlate CTSB and CTSL expression with proinflammatory cytokines (CCL-2, IL-6 and IL-1 β).

Patients and Methods: A total of 12 patients without obesity (NOB) and 51 patients with obesity (OB) were involved in this study. Omental AT was collected from all the participants for RNA extraction. Expressions of CTSB, CTSL and proinflammatory cytokines (CCL-2, IL-6 and IL-1 β) were qualified with qRT-PCR. BMI (body mass index) and metabolic parameters were measured.

Results: The mRNA expression levels of both CTSB and CTSL were upregulated in the OB group ($t = 2.693, P < 0.05$; $t = 2.849, P < 0.01$) and were related to TC levels (Std. $\beta = 0.443, P < 0.05$; Std. $\beta = 0.439, P < 0.05$). However, only the CTSB level was related to BMI (Std. $\beta = 0.261, P < 0.05$). In multiple regression analysis, CTSL was independently associated with CCL-2, IL-6 and IL-1 β levels (Std. $\beta = 0.352-0.462, P < 0.05$).

Conclusion: CTSB and CTSL gene expressions were elevated in the omental AT of OB group. CTSL, but not CTSB, was positively correlated with proinflammatory cytokines independently, suggesting that the dysregulation of CTSL may play a significant role in the inflammatory process.

Keywords: adipose tissue, cathepsin, cytokines, obesity, inflammation

Introduction

The prevalence of obesity is high, and it has been rising rapidly in the past few decades. Thus, it has become a serious public health problem globally.^{1,2} Obesity has increased significantly in the past four decades in China, and the latest national prevalence estimates for 2015–19 were 34.3% for overweight and 16.4% for obesity in adults (≥ 18 years).³ Obesity-induced inflammation signaling pathways by increasing proinflammation factors,⁴ leading to insulin resistance.^{5–7} Beyond the storage of excessive energy, adipose tissue (AT) is implicated in the secretion of proinflammatory cytokines, such as interleukin-6 (IL-6), interleukin-1 β (IL-1 β), C-C motif chemokine 2 (CCL-2)/monocyte chemoattractant protein-1 (MCP-1), resulting in persistent and continuous low-grade inflammation.^{6,8}

Autophagy is a recycling system that acts to control intracellular homeostasis, and it is believed to be important for metabolic homeostasis.⁹ It also plays an important role in regulating the levels of inflammatory molecules, such as IL-1 β , CCL-2 and IL-6(6). However, dysregulation of autophagy with lysosomal dysfunction has been reported in the adipose tissue of obese rodents.¹⁰ Lysosomal degradation is the final step of autophagy, and it depends on digestive enzymes contained in lysosomes,¹¹ therefore lysosomal dysfunction may lead to autophagy deflection and contribute to multiple pathogenesis related to obesity.¹²

Lysosomes contain more than 50 hydrolytic enzymes, such as proteases, lipases and nucleases, that are critical for autophagic degradation. CTSB (cathepsin B), CTSL (cathepsin L) and CTSD (cathepsin D) are the most abundant lysosomal proteases.^{13,14} CSTB activation was discovered in both cultured adipocytes exposed to saturated fatty acids in vitro and adipocytes from obese mice fed with high fat diet in vivo, and it was associated with increased inflammatory factors.¹⁵ In rodent studies, CTSL and CTSB protein levels were increased in epididymal fat depot but not in inguinal fat depot. Inhibiting CTSB or CTSL activity reduced the secretion of LPS-induced MCP-1 and IL-1 β expression in macrophages.¹⁶ Recently, CTSB and CTSL expression in overweight/obesity human subcutaneous adipose tissue (SCAT) was reported. This finding indicates that CTSB expression is related to markers of insulin resistance independent of adiposity, and CTSL has been reported to be related to BMI and WHR (waist/hip ratio).¹⁷ Visceral adipose tissue is believed to play a key role in obesity-related inflammation,^{18,19} but few studies have focused on cathepsins expression.

In this study, we performed a cross-sectional analysis and focused on the expression of cathepsins in omental AT, which is a well-known type of visceral AT. We explored the expression of CSTB and CSTL in omental AT and assessed their relationships with inflammatory cytokines to understand the inflammatory process in adipose tissues of individuals with obesity and to provide a target for the prevention or treatment of obesity-related pathology.

Methods

Study Population

The study included 63 individuals who were divided into a group without obesity (NOB, n = 12) and a group with obesity (OB, n = 51) according to their body mass index (BMI, ratio of body weight (kg) to height (m²)). Inclusion criteria for OB group: all the participants met the criteria for weight loss surgery (BMI > 32.5 kg/m² or \geq 30 kg/m² with at least one comorbidity).²⁰ Inclusion criteria for NOB group: patients had cholecystectomy for gallbladder polyps or cholelithiasis, and BMI < 30 kg/m².²¹ Exclusion criteria: patients had diseases that presented with wasting syndrome (malignant tumors, tuberculosis, etc.), patients with acute infectious diseases (pneumonia, acute cholecystitis, peritonitis, etc.), patients with cardiovascular diseases, patients who had taken any drugs that may affect glucose metabolism or inflammation processes in the previous 3 months and patients who smoked or abused alcohol/drugs. The study protocols were approved by the Ethics Committee of Yan'an Hospital Affiliated to Kunming Medical University (number 2021-073-01). Written informed consent for inclusion in the study was collected from each participant in advance.

Anthropometric and Metabolic Parameters

Anthropometric phenotyping was performed according to a previously described method.¹⁷ Peripheral blood was drawn in the morning after fasting for at least 8 hours for laboratory analysis, including fasting glucose levels, lipid profile and fasting insulin levels. Glucose levels, glycosylated hemoglobin (HbA1c) levels and lipid profiles including plasma triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and total cholesterol (TC) levels were measured using Auto-biochemical Analyzer (Olympus, Japan) by standard techniques. Insulin was detected by chemiluminescence with an auto-biochemical immune analyzer (Roche, Japan). For the assessment of insulin resistance, homeostatic model assessment of insulin resistance (HOMA-IR) indices was calculated as $\text{HOMA-IR} = \text{fasting insulin } (\mu\text{U/L}) \times \text{fasting glucose } (\text{mmol/L}) / 22.5$.²²

Collection of Omental Adipose Tissue

A total of 0.5–1 cm³ of omental AT were taken from the omentum by laparoscopy during the surgery and washed with cold saline immediately. After the blood vessels were removed, the collected adipose tissue was stored at -80°C .

Adipose Tissue mRNA Analysis

Total RNA was extracted from adipose tissue samples using the TRIzol method,²³ and 500 ng of total RNA was reverse-transcribed using a SuperRT cDNA synthesis kit (CW BIO). Then, real-time PCRs were performed using an UltraSYBR Real Time PCR detection system (Roche). Reactions were performed in a total volume of 20 μL containing 1.5 μL cDNA, 12.5 μL ultra SYBR green supermix (CW BIO) and gene-specific primers. The following primers were used: CTSL (Forward Primer AAGTGGAAGGCGATGCACAA, Reverse Primer AAAGCCATTCATCACCTGCC), CTSB (Forward Primer TCGGA

TGAGCTGGTCAACTA, Reverse Primer AGCTTCAGGTCCTCGGTA AAA), CCL-2 (Forward Primer AGTTCAGCACAC CAACCTTC, Reverse Primer GGGTCAGCACAGATCTCCTT), IL-6 (Forward Primer AGGTACCAACTTGTCGCACTC, Reverse Primer GCCCTGAGAGTACCTTTCCC), and IL-1 β (Forward Primer GAAATGCCACCTTTTGACAGTG, Reverse Primer TGGATGCTCTCATCAGGACAG). The 18s gene was used as a housekeeping gene and data were calculated using the delta CT method.

Statistical Analysis

IBM SPSS statistics 23 was used to perform statistical analysis. Data are presented as mean \pm standard deviation (SD) unless indicated otherwise. To compare group difference, the χ^2 test was used for categorical variables, the independent samples *t* test was used for continuous variables with normal distribution and the Mann–Whitney *U*-test for continuous variables with skewed distribution. Variables with skewed distribution were ln-transformed, and Pearson's correlation and stepwise multiple linear regression were used to identify associations between different variables. For all analyses, *p* value <0.05 was considered significant.

Results

Demographic and Clinical Characteristics of the Study Population

The clinical characteristics of the participants in the two groups are shown in Table 1. The age of the participants ranged from 23 to 40 years old. Compared to individuals without obesity, individuals with obesity had higher BMI, plasma LDL, fasting glucose, HbA1c, and HOMA-IR values. There was no significant difference in age or sex between the two groups.

The Gene Expression of CCL-2, IL-6 and IL-1 β Was Elevated in the Adipose Tissue of Individuals with Obesity and Was Associated with BMI and HOMA-IR

The gene expression of CCL-2, IL-6 and IL-1 β was elevated in the omental AT of OB group ($t = 3.972$ – 5.079 , $p < 0.05$) and was positively associated with BMI and Ln (HOMA-IR) (Figure 1). After adjusting BMI, only CCL-2 was associated with Ln (HOMA-IR) ($r = 0.279$, $p = 0.039$).

CTSB and CTSL Gene Expression Was Elevated in the at of the OB Group

CTSB and CTSL mRNA expression of the omental AT in the OB group was elevated compared with that in the NOB group ($t = 2.693$, $p < 0.05$; $t = 2.849$, $p < 0.01$). A strong positive relationship between CTSB and CTSL mRNA expression was observed ($r = 0.836$, $p < 0.01$) (Figure 2). CTSB mRNA expression was positively associated with BMI, TG, TC and LDL levels. CTSL mRNA expression was positively associated with TG, TC and LDL (Table 2).

Table 1 Demographic and Clinical Characteristics of Study Population

	NOB	OB	t/Z/ χ^2 -value	P-value
Participants	12	51		
Gender (F/M)	5/7	34/17	2.575	0.109
Age (years)	31.92 \pm 6.90	33.29 \pm 5.28	-0.298	0.766
BMI (kg/m ²)	24.39 \pm 1.85	36.10 \pm 4.62	-13.977	<0.001
Fasting glucose (mM)	4.94 \pm 0.71	6.02 \pm 2.24	-2.293	0.022
Fasting insulin (mU/L)	9.90 \pm 3.73	27.85 \pm 18.52	-3.406	0.001
HOMA-IR	2.11 \pm 0.60	7.95 \pm 7.85	-5.024	<0.001
HbA1c (%)	5.25 \pm 0.34	5.81 \pm 1.00	-3.406	0.001
TG (mM)	1.98 \pm 1.44	2.59 \pm 3.25	-0.945	0.345
TC (mM)	4.51 \pm 0.69	4.87 \pm 0.95	-1.103	0.270
LDL (mM)	2.53 \pm 0.62	3.03 \pm 0.72	-2.210	0.031
HDL (mM)	1.12 \pm 0.21	1.12 \pm 0.20	-0.063	0.950

Notes: Data given as n (participants) or mean \pm SD.

Abbreviations: F, female; BMI, body mass index; HOMA-IR, Model Assessment of Insulin Resistance; HbA1c, glycated hemoglobin; TG, triglycerides; TC, total cholesterol; LDL, low-density lipoproteins; HDL, high-density lipoproteins. NOB, patients without obesity; OB, patients with obesity.

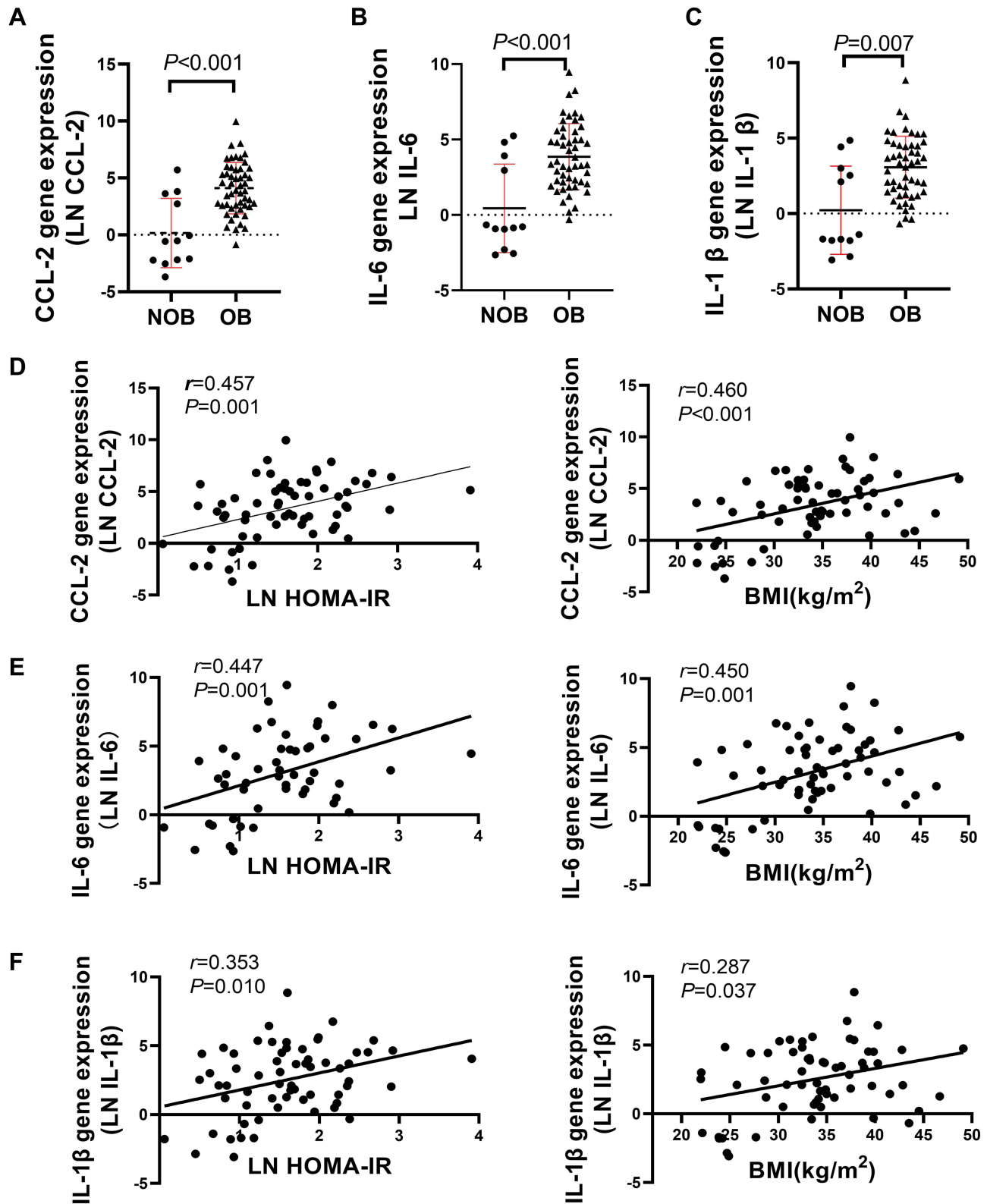


Figure 1 Increased adipose tissue associated CCL-2, IL-6 and IL-1β gene expression in OB group. (A-C) qRT-PCR analysis for CCL-2, IL-6 and IL-1β of omental adipose tissue from NOB and OB groups. (D-F) CCL-2, IL-6 and IL-1β gene expression are respectively positively associated with BMI and Ln(HOMA-IR).

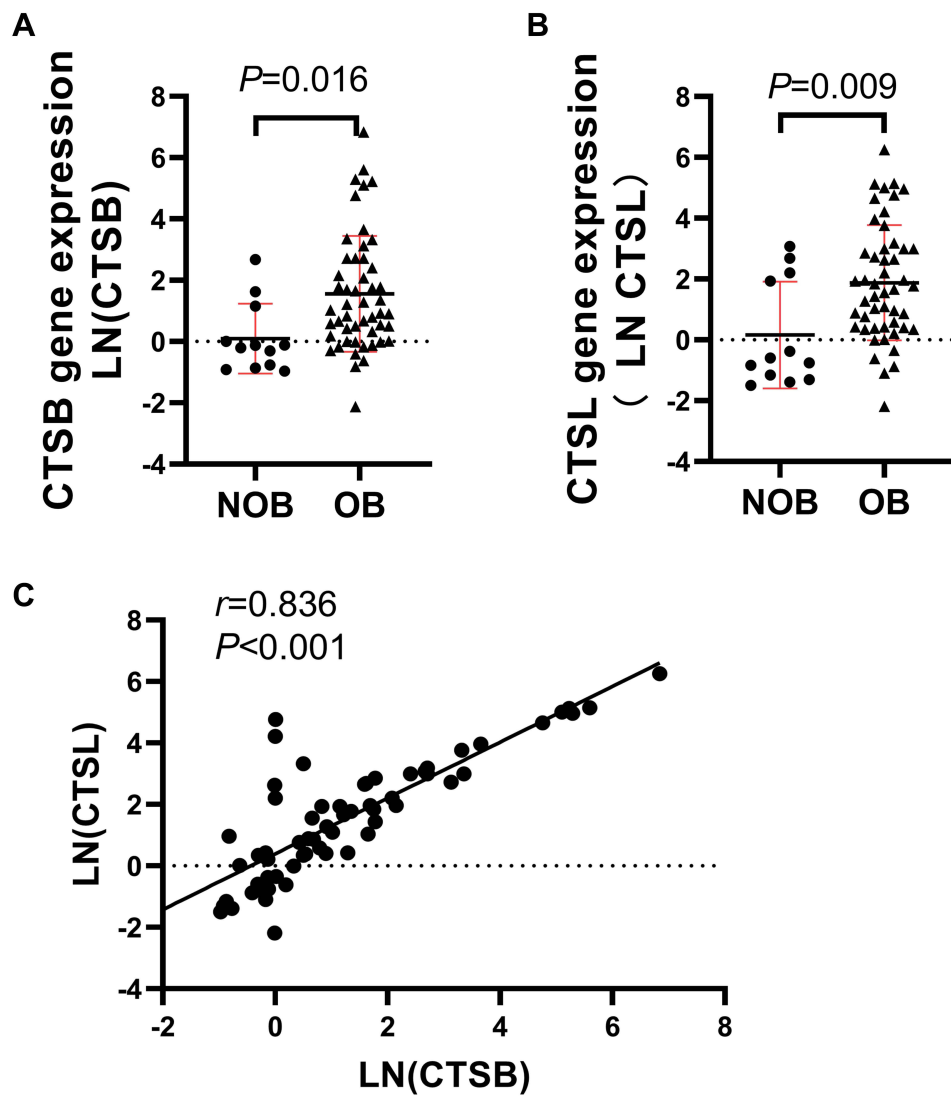


Figure 2 Increased CTSB and CTSL gene expression in omental adipose tissue of obese individuals. (A-B) qRT-PCR for CTSB and CTSL mRNA of omental adipose tissue from NOB and OB group. (C) In the study cohort, CTSL expression correlated positively with CTSB gene expression.

Table 2 Pearson's Correlation Coefficients for CTSB and CTSL Gene Expression and Clinical Characters

	Ln CTSB	Ln CTSL
Age (years)	0.104	0.156
BMI (kg/m ²)	0.283*	0.210
Fasting glucose (mM)	-0.084	-0.056
TG (mM)	0.288*	0.264*
TC (mM)	0.456**	0.439**
HDL (mM)	-0.035	-0.035
LDL (mM)	0.401**	0.368*
HbA1c (%)	0.121	0.105
Ln (HOMA-IR)	0.184	0.170

Notes: * $P<0.05$, ** $P<0.01$. Ln (CTSB), Ln-transformed of CTSB; Ln (CTSL), Ln-transformed of CTSL.

No association was observed between the mRNA expression of CTSB or CTSL and fasting glucose, insulin or HOMA-IR values ($P < 0.05$). To determine the factors that affect CTSB or CTSL gene expression, a stepwise multiple linear regression analysis was performed. With Ln (CTSB) or Ln (CTSL) as dependent variable, BMI, TG, TC and LDL values were included as independent variables. Multiple linear regression analysis showed that BMI and TC levels were retained in the final model with Ln (CTSB) as dependent variable. BMI and TC levels were positively associated with CTSB gene expression and together explained 25.1% of the variance of the observed Ln (CTSB). Only TC levels were retained in the final model with Ln (CTSL) as the dependent variable. TC levels were positively associated with Ln (CTSL) and were responsible for 17.9% of the variance of the observed Ln (CTSL) (Table 3).

CTSL Correlated with Proinflammatory Cytokines

As IL-1 β , IL-6 and CCL-2 might be regulated by autophagy,¹⁸ we wondered whether they were related to CTSB/CTSL expression in the omental adipose tissue of humans. To explore the relationship between CTSB/CTSL and inflammatory factors, stepwise multiple linear regression was performed (Table 4).

Ln (IL-1 β), Ln (IL-6) or Ln (CCL-2) were used as the dependent variables, respectively, and BMI, CTSL, CTSB, TG, TC and LDL levels were included as the independent variables. BMI, TC and CTSL were retained in the final model with Ln (IL-6) or Ln (CCL-2) acting as the dependent variable, which explained the 27.9% and 29% changes in the levels of Ln (IL-6) and Ln (CCL-2), accordingly. When Ln (IL-1 β) was used as a dependent variable, only CTSL was retained in the final model and explained 16.9% change of Ln (IL-1 β). It showed that CTSL, but not CTSB, was positively correlated with the expression of all three proinflammatory factors CCL-2, IL-6 and IL-1 β expression.

Table 3 Relationship Between CTSB, CTSL Gene Expression in Omental Adipose Tissues and Adiposity

Dependent Variables	Ln CTSB			Ln CTSL		
	Std. β	P value	Adj.R ²	Std. β	P value	Adj.R ²
BMI	0.261	0.023	0.251		0.108	0.179
TC	0.443	0.000		0.439	0.000	
TG		0.923			0.824	
LDL		0.648			0.388	

Notes: Stepwise multiple linear regression analysis. Dependent variables: Ln CTSB or Ln CTSL; independent variables: BMI, TC, TG, LDL.

Table 4 Relationship Between Gene Expression Between CTSB/CTSL and Pro-Inflammatory Cytokines in Omental Adipose Tissues

Dependent Variable	Ln CCL-2			Ln IL-6			Ln IL-1 β		
	Std. β	P value	Adj.R ²	Std. β	P value	Adj.R ²	Std. β	P value	Adj.R ²
BMI	0.404	0.001	0.391	0.376	0.001	0.397		0.090	0.165
CTSL	0.352	0.002		0.365	0.002		0.426	0.000	
CTSB		0.091			0.229			0.108	
TG	0.276	0.023		0.290	0.010			0.055	
TC		0.983			0.897			0.161	
LDL		0.284			0.215			0.188	

Notes: Stepwise linear regression analysis. Ln CCL-2, Ln IL-6 and Ln IL-1 β were ln-transformed of CCL-2, IL-6 and IL-1 β respectively. Dependent variables: Ln CCL-2, Ln IL-6 or Ln IL-1 β . Independent variables: BMI, CTSL, CTSB, TG, TC, LDL.

Discussion

CTSB mRNA expression was upregulated in SCAT from obese compared to lean male (21–35 years old) subjects.²⁴ In our study, a similar upregulation of CTSB mRNA expression was observed in omental AT from OB group. Stepwise multiple linear regression showed that both BMI and TC levels were positively correlated with CTSB mRNA expression, while fasting glucose and HOMA-IR were not. However, a recent study reported that CTSB mRNA expression in SCAT was unrelated with BMI or WHR but negatively correlated with whole-body insulin resistance in middle-aged individuals with obesity (age 52 ± 2 years).¹⁷ This difference may be due to different subject characteristics (age and BMI). CTSL mRNA expression was also upregulated in the omental AT of the OB group in our study and was strongly related to CTSB mRNA expression. Different from CTSB mRNA expression, multiple linear regression analysis showed that CTSL mRNA expression was only related to TC. Rodent models indicated that the mutual interaction between CTSB and CTSL in lysosomes maintains lysosomal function.^{12,25} Moreover, CTSL mRNA expression was significantly elevated in the epididymal AT (visceral AT) of mice with either high-fat diet-induced or genetically induced obesity compared with control mice, while the maturation of CTSL declined. The upregulation of CTSB was a compensatory activation of the downregulation of mature CTSL in obese epididymal AT.¹⁰

Interestingly, we found that the mRNA expression of both CTSB and CTSL was related to TC. Autophagy might regulate lipid homeostasis through the selective degradation of lipid droplets via lipophagy.^{26,27} CTSB and CTSL are two of the most abundant cathepsins that play important roles in the degradation process of autophagy, which is a cellular process strongly associated with lysosomal function. As shown by 3T3L-1 adipocytes, CTSB could be linked to increased lipolysis and possible inflammatory responses via reduced perilipin 1 expression.²⁸ Previous studies found HFD feeding selectively increased CTSL and CTSB levels in epididymal fat in mice. CTSB and CTSL modulate cholesterol homeostasis primarily by regulating the expression of genes involved in cholesterol uptake and NPC2-mediated cholesterol cellular trafficking in macrophages.¹⁶ In a study of intestine tissue, CTSB was discovered to have an effect on cholesterol absorption from the intestine.²⁹ All these studies suggest a possible relationship between cholesterol metabolism and CTSB/CTSL. However, more research is needed to clarify the interplay between cholesterol and lysosomal enzymes.

IL-1 β , IL-6 and CCL-2 are proinflammatory factors that are known to induce insulin resistance and lead to the development of T2DM.^{8,18} In the present study, the mRNA expression of IL-1 β , IL-6 and CCL-2 in the omental AT was increased in the OB group compared to NOB group. It was also positively associated with BMI and insulin resistance, which is similar to the results of previous studies.³⁰

IL-1 β is secreted by NLRP3 (NLR family pyrin domain containing 3) activation and promotes insulin resistance.^{31,32} Autophagy plays an important role in the regulation of NLRP3 activation,³³ and inhibition of autophagy increased IL-1 β secretion.³⁴ Epididymal adipose tissue from mice with HFD-induced or genetically induced obesity displayed lysosomal abnormalities, including the deteriorated CTSL function, compensatory elevations in CTSL mRNA and the activation of CTSB, and the lysosomal abnormalities caused NLRP3 activation.¹⁰ However, in some studies, CTSL was found to be involved in NLRP3 activation, but autophagy levels were not assessed. Additionally, CTSB and CTSL were implicated in ischemia/reperfusion (I/R)-triggered NLRP3 inflammasome activation in renal tissue, and downregulation of CTSB and CTSL attenuated this activation.³⁵ CTSL knockout cells released significantly less IL-1 β , indicating a unique role of CTSL in NLRP3 activation.^{36,37} Tang et al showed that the knockdown of cathepsin B, L and D affected NLRP3 inflammasome activation by reducing pro-caspase 1 and pro-IL-1 β levels in the renal tubular epithelial cell line (HK-2).³⁸ In our present study, elevated CTSL mRNA was positively related with CTSB and IL-1 β . This suggested that CTSL dysfunction, the compensatory elevated CTSB, and abnormal autophagy may exist in the omental AT of people with obesity and lead to upregulation of IL-1 β ; however, this hypothesis needs further confirmation.

The present data showed that CCL-2 and IL-6 mRNA expression was also associated with CTSL. IL-1 β , IL-6 and CCL-2 are all NF- κ B-dependent proinflammatory mediators, and autophagy is involved in the degradation of NF- κ B.³⁹ Blocking autophagy increases CCL-2 and IL-6 levels in the stromal vascular fraction (SVF) of both young and old mice.⁴⁰ Inhibition of cathepsin B, S, and L leads to a polarization shift from M2- to M1 macrophages with elevated IL-6, CCL-2 and IL-1 β levels, which is also associated with distinct alterations in lysosomal signaling and lipid metabolism.⁴¹ However, the influence of increased mRNA

expression of CTSL on autophagy in human AT is still unknown. Conversely, inflammatory cytokines may also affect CTSL expression. In human lung epithelial cell lines, IL-6 lead to a twofold increase in CTSL mRNA expression. At the protein level, IL-6 induced increased amounts of CTSL but not CTSB.⁴² Although these studies confirmed the association between CTSL and IL-6/CCL-2, how they interact with each other was elusive. Independent of autophagy, lysosomal membrane permeabilization and cathepsins release also result in increased inflammation.^{43,44} The involvement of cathepsins in inflammasome activation may act as a system that works together and has both compensatory and independent functions.^{38,45}

In the future, it will be necessary to confirm the protein level and activity of CTSL/CTSB and assess autophagy flux in the omental AT of individuals with obesity. It will also be beneficial to understand the influence of CTSB/CTSL on autophagy and inflammation.

Conclusion

CTSB and CTSL expression in the omental AT of individuals with obesity was elevated compared to that in individuals without obesity. CTSL, but not CTSB, was positively independently related to CCL-2, IL-6 and IL-1 β , suggesting that the dysregulation of CTSL may play a significant role in the inflammatory process in AT. However, the present study showed a small cross-sectional analysis, and more research is needed to confirm the relationship and determine the causality of these relationships.

Abbreviations

BMI, body mass index; IL-6, interleukin-6; IL-1 β , interleukin-1 β ; CCL-2, C-C motif chemokine 2; MCP-1, monocyte chemoattractant protein-1; CTSD, cathepsin D; SCAT, subcutaneous adipose tissue; TG, triglycerides; LDL, low-density lipoproteins; HDL, high-density lipoproteins; TC, total cholesterol; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; HbA1c, glycosylated hemoglobin; NLRP3, NLR family pyrin domain containing 3.

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Disclosure

All the authors declared no conflicts of interest in this work.

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