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Original Article

The gene expression of CTRP12 but not CTRP13 is upregulated in both visceral and subcutaneous adipose tissue of obese subjects

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ABSTRACT

Obesity is a well-known chronic low-grade inflammation condition characterized by dysregulated adipokine secretion and function. Both CTRP12 and CTRP13 are adipokines that influence glucose and lipid metabolism. We aimed to investigate CTRP12, CTRP13, and inflammatory gene expressions in subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) from obese women who underwent bariatric surgery in comparison with the normal weight women. This case-control study included 20 obese [body mass index (BMI) > 35–40 kg/m^2] candidates for bariatric surgery and 20 normal-weight women (BMI <25 kg/m^2) as control group, who underwent elective surgeries. Real-time PCR was used to evaluate mRNA expression levels of CTRP12, CTRP13, and inflammatory genes in SAT and VAT from both groups. We observed significantly higher mRNA expression of CTRP12 in SAT ($p = 0.048$) and VAT ($p = 0.046$) from obese patients compared to the controls. There was significantly greater expression of IL-6 and MCP-1 inflammatory genes in SAT ($p = 0.013$ and $p = 0.005$ respectively) and VAT ($p = 0.000$ and $p = 0.001$ respectively) of obese patients compared to the control group. IL-1β ($p = 0.015$) and TNF-α ($p = 0.014$) expressions significantly increased in VAT from obese patients compared to the control group. Spearman correlation analysis showed that CTRP12 expression significantly correlated with obesity indices. Our findings showed that CTRP12 significantly increased in both VAT and SAT of obese group. More importantly, we observed a positive correlation between CTRP12 with inflammatory parameters. These results indicated that CTRP12 might be part of an intricate network for glucose metabolism and obesity-related inflammation processes.

1. Introduction

Obesity is a major public health epidemic worldwide. It is considered to be a chronic low-grade inflammation that has a strong relationship with type 2 diabetes (T2D), insulin resistance, coronary artery diseases (CAD), non-alcoholic fatty liver disease (NAFLD), and other metabolic conditions [1,2]. Adipose tissue recognized as an endocrine organ that affects energy hemostasis and plays a central role in the development of metabolic disorders [3]. Specifically, adipose tissue is classified as fat depots located in the subcutaneous area [subcutaneous adipose tissue (SAT)], abdominal subcutaneous, overall coverage or visceral fat. Mesenteric and omental adipose depots are visceral fat depots located in the abdominal cavity [4]. The anatomical, cellular, molecular, clinical and physiological characteristics of VAT is different from that present in SAT [4]. Adipose tissue is considered a metabolically dynamic organ that secrets biologically active compounds called adipokines [5]. C1q/TNF-related proteins (CTRPs), a group of recently discovered adipokines, are the focus of numerous research studies [6]. CTRP molecules have a highly protected homology

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sequence with adiponectin and possess structural resemblance to TNF-α. Importantly, there is compelling evidence that CTRPs affect both energy metabolism and inflammatory pathways [7].

CTRP12 is an insulin sensitivity factor that is 19% homologous with adiponectin [8,9]. According to a report, CTRP12 enhances insulin sensitivity and hyperglycemia, and reduces inflammation in adipose tissue in DIO mice [10]. Studies on CTRP12 have shown decreased serum levels and CTRP12 expressions in obesity and T2DM whereas improvement of insulin resistance has led to significant increases in CTRP12 levels [8,10,11]. Moreover, in adipose tissue systemic administration of CTRP12 can reduce inflammatory responses via decreasing mRNA expression of proinflammatory genes and macrophage repletion in obese mice [10]. In addition, in adipose tissue CTRP12 is involved in regulating of glucose levels through both insulin-dependent and insulin-independent manner [6]. Despite extensive research on animal models, there is scant information about CTRP12 expression in obese humans.

CTRP13 is another adipokine that vastly secreted by stromal vascular cells as multimeric protein in cerebral and adipose tissue of humans and adipose tissue of mice [12]. Recent studies indicated that CTRP13 has a substantial function in food absorption, body weight balances and modulating of homeostasis. There is evidence that CTRP13 can improve glucose absorption in muscular cells and liver, enhance insulin resistance, and suppress the JNK stress signaling pathway that disrupts routes for insulin signaling [12,13]. In the liver, this adipokine controls *de novo* glucose synthesis by decreasing expression levels of glucose-6-phosphate dehydrogenase (G6PD) and phosphoenolpyruvate carboxykinase. Moreover, our recent study demonstrated decreased serum levels of CTRP13 in NAFLD and T2D subjects compared to healthy group [14].

Up to now, there has been no study that evaluated CTRP expressions in human adipose tissue and their possible association with metabolic indices in the context of obesity. Hence, in an attempt to understand the mechanisms and molecules involved in obesity pathogenesis, we designed the current study to investigate CTRP12, CTRP13, and inflammatory gene expressions in SAT and VAT from obese women compared to a normal-weight group. We also intended to evaluate the possibility of an association of gene expressions with clinical and biochemical factors in obese individuals.

2. Materials and methods

2.1. Study population

This was a case-control study of 20 obese women between 18 and 49 years of age with body mass index (BMI) greater than 35 kg/m^2. The women in obese group were candidates for bariatric surgery. The control group comprised 20 normal-weight women between 18 and 49 years of age who had BMIs less than 25 kg/m^2. We recruited the obese group from the Bariatric Surgery Center at Erfan Hospital and the control group from advanced laparoscopic surgeries centers at Loqman Hakim and Sina hospitals in Tehran, Iran. Women in the obese group were qualified for bariatric surgery (Roux-en-Y gastric bypass and vertical sleeve gastrectomy) under specialist supervision. The normal-weight group consisted of women who underwent elective surgeries (inguinal cholecystectomy, abdominal hernia, and gastric bypass). We excluded anyone with T2DM, NAFDL, CAD, cancer, acute or chronic infectious diseases, autoimmune diseases, thyroid and hormonal dysfunctions, congenital conditions, neurological disorders, or pregnancy. Any patient who underwent surgery during the last 6 months, hospital admission in the last 6 months, or who used weight-loss medications was also excluded. All participants were informed about the research and provided written consent for participation. This research was in line with the Declaration of Helsinki and approved by the local Ethics Committee at Shahid Beheshti University of Medical Sciences (IR.SBMU.RETECH.REC.1396.960).

The specialist collected participants' demographic data collection. After subjects provided their written informed consent, they were weighed with a digital scale that had 0.1 kg accuracy. A laser meter that had an accuracy of 0.1 cm was used to record participants' heights. We calculated BMI by dividing each participant's weight (in kg) by the square of their height (in m). A qualified nurse used a tape measure to determine waist circumference (WC) and hip circumference to calculate the waist-to-hip ratio (WHR). The participants were seated and allowed to rest in a quiet room before obtaining the systolic and diastolic blood pressure readings. Blood pressures were measured 3 times with a sphygmomanometer and the average value was recorded for each participant.

Patients provided venous blood samples after a 12 h fasting period and just prior to surgery. Serum was separated by refrigerated centrifugation at 800×*g* for 10 min, and it was stored at −80 °C until the next analysis. Biochemical analyses were conducted according to standard clinical laboratory procedures at Massoud Clinical Diagnostic Laboratory, Tehran, Iran. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), serum creatinine (Cr), uric acid, total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) were all measured by routine enzymatic methods. Fasting blood sugar (FBS) was measured with a Cobas6000 E501 autoanalyzer with the hexokinase method. We used a SEBIA capillary electrophoresis (CE) instrument (France) to measure the percent of hemoglobin A1c (HbA1c). High sensitivity-reactive protein (hs-CRP) was measured an immunoturbidometric method in a Roche integra analyzer. The fasting insulin (FINS) level was determined according to the ECL method in a Cobas6000 E601 autoanalyzer.

2.2. Tissue biopsy

The surgeon excised approximately 0.5 g of SAT and VAT from each participant at the beginning of the surgical procedure. VAT was obtained from the abdominal cavity. SAT was obtained after the surgeon used a scalpel to create a small incision 0.5 cm deep in the superficial skin. The adipose tissue samples were washed in the cold and sterile phosphate buffered saline and were aseptically divided into small sections and stored in RNase-free tubes. Then, tissues were snap frozen in liquid nitrogen, and immediately stored at −80 °C until needed.

2.3. RNA extraction and real-time PCR

We used 70–100 mg of each sample for RNA extraction. Due to the high lipid concentration and low numbers of adipose cells, the mechanical destruction method was used to obtain adequate levels of RNA. We placed the adipose tissues in a porcelain mortar and added liquid nitrogen. The sample was gently ground with a pestle until it became a homogenized powder. Next, we added QIAzol Lysis Reagent to the homogenized powder for total RNA extraction. RNeasy Lipid Tissue Mini Kit (cat no: 74804, Qiagen, Germany) was utilized according to the manufacturer's instructions. 1 μg of RNA from each sample were reverse transcribed using PrimeScript™ RT (cat: PR037A, Takara Bio, Inc., Japan) reagent kits. Then real-time PCR was conducted for each sample in 20 μl reactions as duplicates by SYBR® Premix Ex Taq ™ II kit (Cat. #RR820L, Takara Bio inc,Japan). Table 1 lists the used primer sequences. We performed data normalization with glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) as the internal control. Relative mRNA expressions were examined by the $2^{-\Delta CT}$ method.

Table 1
Primer sequences used for real-time PCR.

Target gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
CTRP12	CCCACATGACATGGCTGAAC	GCAGCTCCTGAAACTCGTGAAG
CTRP13	GAGTTGTGGTGGTGGCAAAG	TCACCTTTGTCGCCCTTCTC
IL-1β	AAACAGATGAAGTGCTCCTTCC	AAGATGAAGGGAAAGAAGGTGC
IL-6	CCAGCTATGAACTCCTTCTC	GCTTGTTCCTCACATCTCTC
MCP-1	TCAGCCAGATGCAATCAA-TG	ATGGTCTTGAAGATCACAGC
TNF-α	GAAAGCATGATCCGGGACGTG	GATGGCAGAGAGGAGGTTGAC
GAPDH	TGGAAGGACTCATGACCACA	AGGGGTCTACATGGCAACTG

CTRP12: C1q/TNF-related protein-12; CTRP13: C1q/TNF-related protein-13; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; IL-1β: Interleukin 1-beta; IL-6: Interleukin-6; MCP-1: Monocyte chemoattractant protein 1; TNF-α: Tumor necrosis factor-alpha.

2.4. Statistical analysis

Continuous variables were tested for normality by the Shapiro-Wilk test before analysis. Variables with normal distribution are expressed as means ± standard error of the mean (SEM). Variables without normal distribution are displayed as median [interquartile ranges (IQR)]. Between group comparisons were analyzed by the student's t-test and Mann-Whitney *U* test when indicated, based on normality assumption. Spearman's rho correlation coefficients were computed for the associations of genes together and for their associations with clinical variables. Multivariate analysis of covariance (ANCOVA) was performed for the effects of biochemical factors adjustment. We used the comparative CT method (Schmittgen and Livak) for mRNA expression analysis [15]. We calculated $2^{-\Delta CT}$ (relative expression) from the measured ΔCT by real-time PCR. For all analyses, p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS (version 16.0; SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Anthropometric and laboratory parameters

Table 2 shows anthropometric, clinical, and metabolic characterizations of the obese and normal-weight women. Participants had a mean age of 36.5 years for the controls and 35 years for the obese group. We observed significantly higher WC, BMI, and hip circumference measurements in the obese group compared to the normal-weight group ($p = 0.000$). The obese group had higher FINS and HOMA-IR values than the controls ($p = 0.000$). There were significantly higher LDL-C, TC, and hs-CRP values in obese patients ($p < 0.05$). However, age, FBS, ALP, AST, and ALT levels did not show a significant difference between the two groups ($p > 0.05$). Moreover, the indices of kidney function; urea and creatinine were significantly higher in obese group than the controls.

3.2. The mRNA gene expression levels in VAT and SAT

We compared the expressions of CTRP12, CTRP13, and inflammatory genes in VAT and SAT from obese and normal-weight subjects (Figs. 1–3). Fig. 1a and b shows significantly greater CTRP12 expression in SAT ($p = 0.048$) and VAT ($p = 0.046$) in obese patients compared to the control group. However, the mean value of CTRP13 mRNA expression in VAT ($p = 0.25$) and SAT ($p = 0.37$) were similar between the two studied group (Fig. 1 c, d). IL-1β gene expression significantly increased in VAT from obese patients compared to the control group ($p = 0.015$; Fig. 2b). There was significantly elevated IL-6 expression in SAT ($p = 0.013$) and VAT ($p = 0.000$) from the obese group compared to the control group (Fig. 2 c, d). Also MCP-1 gene expressions had a significant elevation in SAT ($p = 0.005$) and VAT ($p = 0.001$) as seen in Fig. 3 a, b. Despite the significant increase in TNF-α expression observed in VAT ($p = 0.014$) in the obese group, there was no significant difference in TNF-α gene expression in SAT ($p = 0.34$) from the obese group compared to the control group (Fig. 3c and d). After ANCOVA adjustment for the effect of HOMA-IR on CTRP12 gene expression, the p-values were attenuated ($p > 0.05$).

We also conducted Spearman's correlation analysis in whole study participants to determine whether mRNA expression of CTRP12 and CTRP13 correlates with inflammatory gene expression and with obesity and anthropometric indices. The results showed that CTRP12 gene expression had a significant correlation with obesity indices (WC, hip circumference, and BMI), HOMA-IR and TNF-α VAT gene expression. However, there was no significant correlation between CTRP12 expression and other inflammatory cytokines (Table 3). CTRP13 gene expression significantly correlated with TNF-α ($r = 0.325$, $p < 0.021$) and MCP-1 expressions ($r = 269$, $p < 0.047$) in SAT.

Table 2
Clinical and laboratory characteristics of the study participants.

	Normal- weight group	Obese group	p-value
Age (years)	36.5 (31–50)	35 (30–38)	**0.115**
Weight (kg)	69.5 (62.75–73)	107 (98–112)	**0.000***
Height (m)	163 (154.5–167.75)	163 (158–166)	**0.42**
BMI (kg/m^2)	24.93 (24.19–25.94)	41 (36.3–45.2)	**0.000***
WC (cm)	88 (85–101)	114 (110–125)	**0.000***
Hip circumference (cm)	101.5 (93.75–107.25)	131 (120–140)	**0.000***
WHR	0.88 (0.87–0.93)	0.9 (0.84–0.94)	**0.494**
SBP (mm Hg)	120 (110–120)	120 (110–122)	**0.379**
DBP (mm Hg)	75 (70–80)	70 (60–80)	**0.32**
FBS (mg/dL)	90(81–98)	85 (81–92)	**0.22**
FINS	7.6 (5.8–8.8)	19.4 (16–24.4)	**0.000***
HOMA-IR	1.64 (0.92–2.09)	3.9 (3.3–5.3)	**0.000***
hs-CRP (mg/dL)	1.68 (1.06–2.16)	4.8 (2.6–9.4)	**0.002***
HDL-C (mg/dL)	44 (38–48)	44.7 (41.2–51.7)	**0.42**
LDL-C (mg/dL)	85 (70–110)	119 (103–131)	**0.001***
VLDL (mg/dL)	20 (14.2–28)	21(17–29)	**0.21**
TC (mg/dL)	137 (115–165)	186 (167–201)	**0.001***
TG (mg/dL)	91 (66–124)	124 (110–156)	**0.06**
HbA1c (%)	5.3 (5.1–5.4)	5.4 (5.1–5.6)	**0.43**
Urea (mg/dL)	20.8 (17–26.9)	25.5 (21.3–28.2)	**0.048***
Cr (mg/dL)	0.57 (0.5–0.65)	0.7 (0.6–0.8)	**0.000***
AST (U/l)	15.8 (11.7–20.95)	20.6 (15.7–23.4)	**0.063**
ALT (U/l)	12.8 (11.1–17.9)	19.5 (14.7–27.8)	**0.082**
ALP (U/l)	63.7 (51.2–83.2)	70.8 (62.2–81.9)	**0.207**

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; BMI: Body mass index; Cr: Creatinine; DBP: Diastolic blood pressure; FBS: Fasting blood sugar; FINS: Fasting insulin; HbA1c: Hemoglobin A1c; hs-CRP: High-sensitivity C-reactive protein; HDL-C: High-density lipoprotein-cholesterol; HOMA-IR: Homeostatic model assessment of insulin resistance; LDL-C: Low-density lipoprotein-cholesterol; SBP: Systolic blood pressure; TC: Total cholesterol; TG: Triglycerides; VLDL: Very low density lipoprotein; WC: Waist circumference; WHR: Waist-to-hip ratio. Data are presented as median [inter-quartile range (IQR)]. *: $p < 0.05$ indicates significant differences between the study groups.

4. Discussion

Adipokines are proteins secreted from adipose tissue that play an influential role in tissue cross-talk to regulate systemic energy metabolism, and maintain blood glucose and lipid balances within the normal physiological range [16]. CTRP12 and CTRP13 are adipokines primarily secreted from adipose tissue that play a role in glucose and lipid metabolism, and inflammatory responses. Pathological conditions like obesity and T2D could impact CTRP12 and CTRP13 mRNA expression levels [6,7]. It is important to understand the regulatory roles of CTRP12 and CTRP13 in both regulating metabolism and tissue cross-talk and determine the mechanisms involved in obesity and inflammation. To the best of our knowledge,

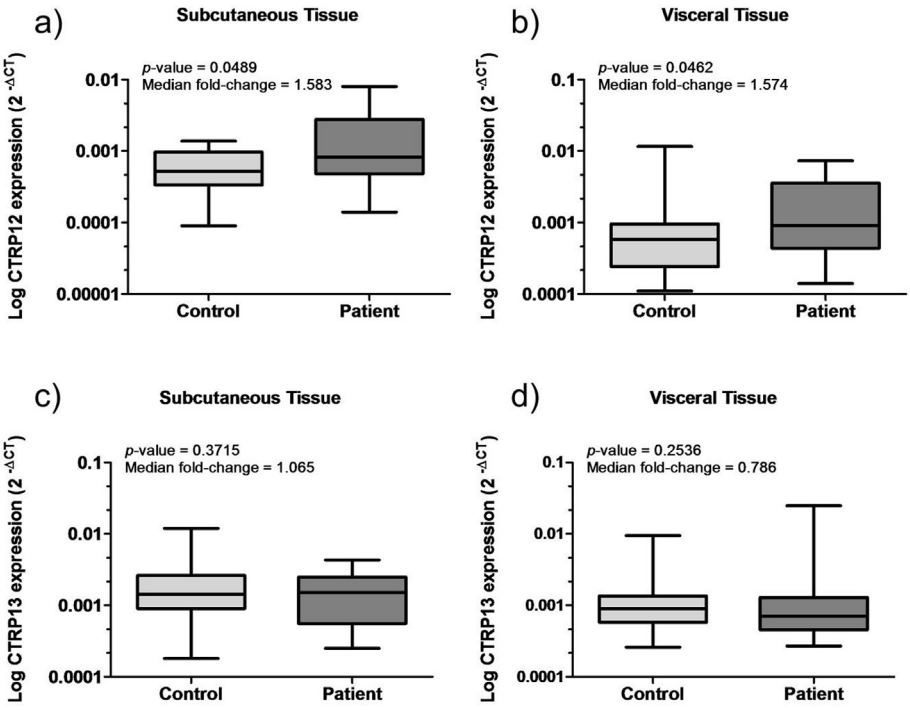

Fig. 1. C1q/TNF-related protein-12 (CTRP12) and CTRP13 mRNA expression levels in subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) samples from the lean and obese groups. (a, b) In obese patients, CTRP12 expression level in SAT ($p = 0.048$) and VAT ($p = 0.046$) were significantly higher compared to the lean group. (c, d) We observed no significant differences in CTRP13 expression in SAT ($p = 0.37$) and VAT ($p = 0.25$) in the obese group compared to the control group. *: $p < 0.05$ indicates significant differences.

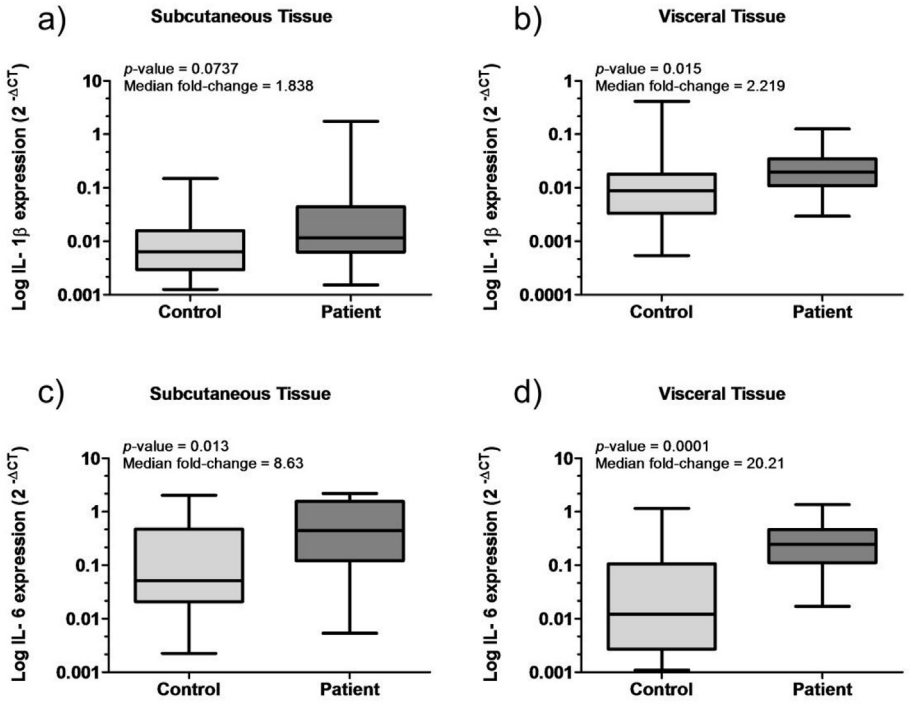

Fig. 2. mRNA expression levels of interleukin (IL)-1β and IL-6 inflammatory cytokines in subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) samples from the lean and obese groups. (a) There were no significant differences found in IL-1β gene expression between obese and control groups in SAT samples ($p = 0.07$) (b) IL-1β gene expression was significantly elevated in VAT samples from the obese group compared to the lean group ($p = 0.015$). (c, d) There was significantly increased mRNA expression level of IL-6 in SAT ($p < 0.013$) and VAT ($p = 0.000$) samples from the obese group compared to the lean group. *: $p < 0.05$ indicates significant differences.

no study has been made to evaluate adipose tissue mRNA expressions of CTRP12 and CTRP13 and their possible correlation with inflammation-related gene expression and metabolic indices in the context of obesity in human.

It has been recently reported that pathological conditions related to obesity such as inflammation and cell stress may

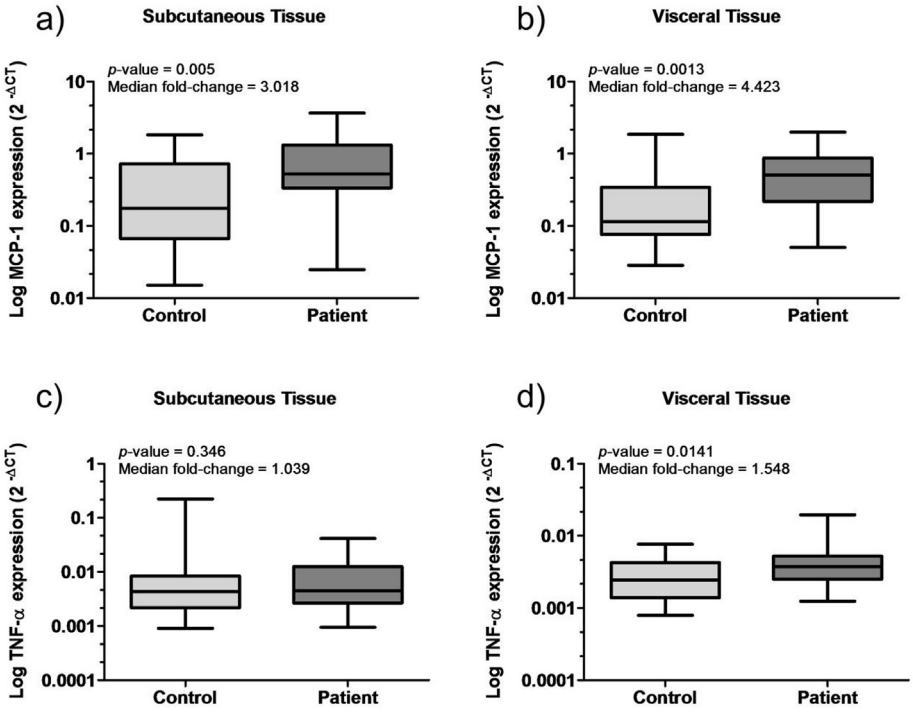

Fig. 3. mRNA expression levels of the monocyte chemoattractant protein 1 (MCP-1) and tumor necrosis factor-alpha (TNF-α) inflammatory cytokines in subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) samples from the lean and obese groups. (a, b) In both VAT and SAT samples, MCP-1 mRNA level was significantly higher in the obese group compared to the control group in the VAT ($p = 0.005$) and SAT samples ($p = 0.001$). (c) No significant differences existed in TNF-α gene expression in SAT from the obese and control groups ($p = 0.3$). (d) There was a significantly higher TNF-α gene expression in VAT samples of obese patients ($p = 0.014$) compared to the control group. *: $p < 0.05$ indicates significant differences.

Table 3
Correlation between CTRP12 gene expression level with anthropometric and laboratory variables.

Variable	CTRP12 gene expression	
	SAT	VAT
BMI (Kg/m^2)	r = 0.257 p = 0.054	r = 0.311 p = 0.025*
WC(cm)	r = 0.377 p = 0.008*	r = 0.265 p = 0.049*
Hip circumference (cm)	r = 0.317 p = 0.023*	r = 0.364 p = 0.014*
HOMA-IR	r = 0.346 p = 0.014*	r = 0.375 p = 0.009*
TNF-α (SAT)	r = 0.104 p = 0.261	r = 0.108 p = 0.253
TNF-α (VAT)	r = 0.108 p = 0.253	r = 0.532 p = 0.000*

BMI: Body mass index; CTRP: C1q/TNF-related protein; HOMA-IR: Homeostatic model assessment of insulin resistance; SAT: Subcutaneous adipose tissue; TNF-α: Tumor necrosis factor-alpha; VAT: Visceral adipose tissue; WC: Waist circumference. Spearman correlation was used to calculate associations between variables. *: $p < 0.05$ indicates statistical significance.

influence CTRP12 and CTRP13 circulation, and mRNA expression levels [8,10,12]. Our results showed significantly increased CTRP12 expression levels in SAT and VAT from obese women.

CTRP12 functions mostly through insulin-independent pathways. CTRP12 might suppress gluconeogenesis and enhance glucose uptake in cultured adipocytes and hepatocytes via activation of the PI3K-Akt signaling pathway [8]. However systemic administration of CTRP12 might be associated with improved insulin signaling in liver and fat tissues, which led to increased insulin sensitivity (insulin-dependent pathway) in Ob/Ob and diet induced obesity (DIO) mice [10]. Tan et al. observed significantly lower CTRP12 serum and mRNA expression levels in fat tissue of DIO mice [17]. Moreover, In a study on lean healthy subjects, Tan et al. reported an increase in CTRP12 serum levels upon inducing hyperinsulinemia by glucose infusion. This finding is in line with our results but in Tan et al. study an acute condition was assessed [11]. Enomoto et al. reported that CTRP12 expression levels in adipose tissue of obese mice were considerably low. In DIO mice, systematic injection of CTRP12 increased insulin sensitivity [10]. Wei et al. noted decreased CTRP12 mRNA levels in obese mice, which increased with administration of anti-diabetic rosiglitazone [8]. The disparity between the our study results and those reported by Enomoto et al. and Wei et al. could be attributed to the sample differences and use of DIO mice instead of obese female subjects in their studies. An intrinsic difference in CTRP12 regulation exists between mice and humans because of the difference in expression levels of CTRP12 and the relative adipose tissue-restricted expression pattern of CTRP12 in humans [18]. There is report that in obese subjects with a large and longlasting fat excess, the down-regulation of lipogenic genes could act as adaptive response to control fat mass development [19]. Since in our study BMI of all obese women are greater than 35 kg/m^2, a possible explanation for increased CTRP12 gene expression could be that it is a defense mechanism against insulin resistance in the obese state. Mechanistically, It can be the effect of CTRP12 on AMPK pathway which in turn lead to suppress lipogenesis pathway [20,21]. In addition, the current study has only included females, whereas the mice model studies included both male and female mice. Because of the lack of human studies on CTRP12 mRNA expression levels in adipose tissue, we could not compare the results of this study with similar studies.

Reports from several *in vitro* studies showed that enhanced AMPK signaling led to stimulated glucose uptake by CTRP13 in adipocytes, myotubes, and hepatocytes and might have a role in

glucose metabolism and improved fatty acid-induced insulin resistance by suppression of the JNK signaling pathway [6,12,22]. In a previous study, we observed an association between CTRP13, a novel adipokine, with T2DM in humans. The T2DM patients expressed significantly less CTRP13 [14]. Wei et al. reported significantly increased CTRP13 mRNA expression in obese mice [12]; however, in the current study, we did not observe any significant CTRP13 expression levels in SAT and VAT from the obese group. An association between biochemical parameters and CTRP13 has been proposed [18,23,24]. Chen et al. reported that serum and mRNA expression of CTRP13 did not significantly differ in T2DM patients compared with a control group. Rather, CTRP13 levels had a significant correlation with BMI, TG, and the TG/HDL ratio [18]. On the other hand, An et al. observed significant differences in CTRP13 levels in NAFLD patients compared to the normal group, which correlated with increased AST, ALT, and TG levels in these patients [23]. We did not find any significant association between CTRP13 mRNA expression levels with TC and LDL-C in the current study. Fadaei et al. reported that circulating levels of CTRP13 showed no correlation with lipid profile [25]. Shanaki et al. showed that CTRP13 had no association with metabolic factors, but was associated with risk of NAFLD in patients with T2DM [24]. Further investigations would be needed to clarify the relationship between CTRP13 and the above-mentioned parameters.

Obesity as a well-known chronic inflammatory condition is linked to the development of insulin resistance. It is generally accepted that secretion of pro-inflammatory adipocytokines such as TNF-a, MCP-1 and IL-1β from macrophages induces insulin resistance in obese state. TNF-a is primarily secreted by adipose tissue-resident macrophages and could promote inflammation in a MAPK and NFκB –dependent pathways. CTRP12 has an anti-inflammatory effect and could alleviate insulin resistance mediated by downregulation of inflammatory cytokines in adipose tissue [8,10,26,27].

Here, we observed CTRP12 gene expression dramatically correlated with TNF-a gene expression in adipose tissue. One possible reason for this finding is that CTRP12 has been increased to reduce TNF-a expression in adipose tissue of obese subjects, which can be a compensatory mechanism for inflammation in adipose tissue. Since CTRP12 is produced by adipocytes, it may be able to reduce insulin resistance through its ability to inhibit macrophage activity in adipose tissue by acting as paracrine manner. However, this hypothesis needs to be precisely investigated.

We acknowledge a number of limitations to our study. Since our study has a cross-sectional design, we cannot deduce any causal relationships between CTRPs gene expression with inflammatory genes and obesity indices. In addition, the assessment of the visceral and subcutaneous abdominal thickness could predict the development of an unfavorable metabolic profile in obesity [28]. Sex difference is another important factor in evaluating the risk of metabolic diseases and must be considered as an independent variable in human and animal studies [29]. Sex can be an influential determinant in insulin resistance, body fat accumulation, and energy metabolism [30]. Therefore, studies with higher sample size on both genders are warranted to unravel the role of CTRPS in obesity and related disorders.

5. Conclusion

Collectively, our findings showed that CTRP12 significantly increased in both VAT and SAT of obese group. More importantly, we observed a positive correlation between CTRP12 and CTRP13 with inflammatory parameters. According to our results and other studies, it is tempting to speculate that CTRP12 and CTRP13 could improve glucose tolerance and insulin sensitivity, and inhibit macrophage-mediated inflammation in adipose tissue. In addition, it seems likely that CTRP12 and CTRP13 can be considered as the parts of a complex network in metabolism and obesity-related inflammation processes. Despite recent endeavors, we lack the necessary knowledge to explain the CTRP12 and CTRP13 mechanisms and their role in obesity and obesity-related complications. Thus, there is an imminent need for more clinical studies to clarify the link between CTRP12 and CTRP13 with glucose and insulin metabolism and obesity-related inflammation.

Conflicts of interest

We declare that we have no conflicts of interest.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Authors contribution

Conceptualization of the project: Mehrnoosh Shanaki.
Project administration: Mehrnoosh Shanaki, Solaleh Emamgholipour.
Supervision: Ali Rahimipour, Mehrnoosh Shanaki.
Laboratory procedures: Abolfazl Omidifar, Karamollah Toolabi.
Data curation: Karamollah Toolabi, Ali Rahimipour.
Formal analysis: Solaleh Emamgholipour, Mehrnoosh Shanaki.
Funding acquisition: Ali Rahimipour.
Investigation: Ali Rahimipour.
Methodology: Karamollah Toolabi.
Writing – original draft: Abolfazl Omidifar.
Writing – review & editing: Abolfazl Omidifar, Mehrnoosh Shanaki, Solaleh Emamgholipour.
All authors read and approved the manuscript.

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