Research Article

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Downregulation of MMP-2 and MMP-9 genes in obesity patients and their relation with obesity-related phenotypes

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Abstract

Objectives: Adipose tissue mediates various bioactive molecules and cytokine discharge. The anti-inflammatory cytokine, interleukin-10 (IL-10), has roles in systemic inflammation. Matrix metalloproteinases (MMPs) are endopeptidases implicating in tissue remodeling, and extracellular matrix degradation. Interleukins and MMPs may have specific roles in obesity development. In this investigation, we marked the roles of IL-10, MMP-2, and MMP-9 in obesity and its related clinical phenotypes.

Methods: Using real-time quantitative polymerase chain reaction (RT-qPCR), also ELISA, IL-10, MMP-2, and MMP-9 mRNA and protein levels were detected respectively in the subcutaneous adipose tissues of 34 patients with obesity and 36 healthy individuals.

Results: *MMP-2* and *MMP-9* gene expression were significantly downregulated in obesity patients compared to controls (p=0.004, p=0.045). Nevertheless, *IL-10* was elevated in the obesity group as to controls (p=0.010). *MMP-2* mRNA expression was correlated with fasting blood glucose levels (r=0.426, p=0.013) in the patient group. As for protein levels, MMP-2 concentration decreased in patients compared to controls (p=0.001). Moreover, MMP-2 was correlated with BMI (r=−0.411; p=0.022) and weight (r=−0.381; p=0.034) in obesity group.

Conclusions: *MMP-2*, *MMP-9*, and *IL-10* may be related to increased susceptibility to obesity development and its related phenotypes in a sample of Turkish patients with obesity.

Keywords: adipose tissue; interleukin-10; MMP-2; MMP-9; mRNA levels; obesity.

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Introduction

Obesity is a common and complex multifactorial disease that can be affected by genetic and environmental factors. Obesity is characterized as a very large amount of body fat or adipose tissue against lean body mass [1]. Metabolic problems in obesity due to the increase of adipose tissue, lead to various conditions like type 2 diabetes, hypertension, dyslipidemia, and coronary heart disease (CAD). Adipose tissue, having intricate connections, is an essential and metabolically dynamic organ, along with fat-soluble vitamins. It has also been shown to have autocrine, paracrine, and endocrine effects. It also has

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functions including energy storage, thermogenesis, and physical protection [2]. Obesity is also described as a low-grade chronic inflammatory process [3]. Although the general assumption is that inflammation is a result of obesity, it is suggested that obesity is a consequence of inflammatory disease [3]. Interleukins (ILs) and matrix metalloproteinases (MMPs) were investigated in the mechanism of obesity in various studies [4].

Matrix metalloproteinases have a major work in extracellular matrix (ECM) degradation and its elements like elastin, laminin, collagens, proteoglycans, fibronectin, and various glycoproteins [5]. MMP-2 has proteolytic activity against matrix and non-matrix proteins, especially against basement membrane components. The main role of MMP-2 is to destroy type IV collagen. It has been reported that tissue breakdown by MMP-2 has a significant role in the mechanism of inflammation [6]. MMP-2 has also a functional role in obesity [7]. MMP-2, together with MMP-9 takes a major part in adipose tissue growth and expanded adipose tissue mass [8]. MMP-9 is both responsible for ECM degradation and cytokine activation. Moreover, it has been reported that MMP-9 was associated with hypertension, cardiovascular diseases (CVD), and obesity [9–11]. Several cytokines and growth factors are also involved in the expression of MMP regulation [12].

Cytokines bind to specific receptors and can differentiate their target cell behavior. Cytokines can also mediate immune reactions, inhibit cell growth, exhibit cytotoxic activity, and trigger or prevent the generation of other cytokines. IL-10 which is an anti-inflammatory cytokine was first identified as a cytokine synthesis inhibitory factor highly activating macrophage/monocyte function and reducing pro-inflammatory cytokine production. IL-10 can have critical roles in obesity development [13].

Inflammatory diseases display the upregulation of the cytokines and also downstream proteases including MMPs. The relationship between MMPs and ILs was shown in several studies [14–16]. In a study, the anti-inflammatory cytokine IL10 was shown to suppress MMP-2 formation by the activation of transcription factor 3 and bind to the MMP-2 gene cAMP response element [16]. Furthermore, another study demonstrated IL-10 considerably suppressed MMP-9 discharge from the PBMCs in control subjects of the study performed in hyperhomocysteinemic subjects. Besides, they observed that IL-10 augmented TIMP-1 levels, the inhibitor of MMP-9, in PBMCs both in hyperhomocysteinemia and control subjects [17]. So far, no studies have questioned the connection between MMP-2, MMP-9, and IL-10 levels together in the adipose tissues of patients with obesity. In the light of the previous studies, since inflammation is a critical risk factor for the formation and progress of obesity, we speculated that MMP-2, MMP-9, and IL-10 might together have roles in obesity development and its related clinical phenotypes. So, we investigated whether MMP-2, MMP-9 and IL-10 mRNA and protein levels were associated with obesity in the adipose tissues of Turkish patients with obesity and evaluated their association with obesity development.

Materials and methods

Subjects and study plan

This investigation was performed according to the Declaration of Helsinki and was agreed upon by the local Ethics Committee of Cerrahpasa Medical Faculty (Ethics Committee issue #40791). The aim of the study was clarified to all individuals, and they were asked to sign a written informed consent form. 34 patients with obesity (11 men, 23 women) and 36 healthy controls (21 men, 15 women) were included in this study. Subcutaneous adipose tissue samples from the abdomen wall taken from patients diagnosed with obesity and who experienced bariatric surgery in the Department of General Surgery, Istanbul Training and Research Hospital were enrolled in this research. Patients were selected according to body mass index (BMI) measurements depending on age and sex. BMI was calculated as follows: BMI (kg/m^2) = Weight (kg)/Height^2 (m^2). While people who have obesity, and have not received any treatment during the illness, such as chemotherapy and medication, were included in the patient group, people having chemotherapy or radiation treatment, those with acute infection, women with pregnancy or lactating, and those who have been declared under other clinical trials were excluded from the study.

Subcutaneous adipose tissue samples were taken from the individuals who did not have obesity and any other diseases leading to obesity. Normal weight subjects who underwent liposuction surgery due to their local excess fat in the Department of Plastic Reconstructive and Aesthetic Surgery, Dr. Lutfi Kirdar Kartal Training and Research Hospital were enrolled in the control group. Exclusion criteria were having a BMI ≥25, any heart disease, diabetes, cancer, severe kidney disease, and pregnancy.

Anthropometry

On the day of surgery, the weight, height, body composition measurements, and blood pressure values of patient and control groups were recorded. Biochemical parameters like total cholesterol, triglycerides, fasting blood glucose, low-density lipoprotein cholesterol (LDL-cholesterol), high-density lipoprotein cholesterol (HDL-cholesterol), fasting insulin, and HbA_{1c} were examined in the individuals of each group. BMI was used to assess obesity.

Adipose tissue collection

Tissue samples taken from patients and controls during surgery with surgical procedures were immediately stored at −80 °C until processing for tissue homogenization and RNA purification. Tissue homogenization, RNA extraction from the adipose tissue samples,

cDNA synthesis, real-time quantitative polymerase chain reaction (RT-qPCR), Lowry method, and enzyme-linked immunosorbent assay (ELISA) were then conducted.

RNA purification

Total RNA was purified using a commercial kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA) as stated in the manufacturer's instructions. After RNA isolation, cDNA synthesis was performed.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Following RNA extraction from the tissue samples, 100 ng of total RNA was reverse-transcribed into complementary DNA (cDNA) with random hexamers as primers by a commercial kit (Roche Diagnostics GmbH, Germany), as stated in the manufacturer's protocol. *MMP-2*, -9, and *IL-10* mRNA expression were detected by RT-qPCR using LightCycler® 1.5 (Roche AppliedBiosystems, California, USA) with the TaqMan method. ACTB was used as an endogenous control for the normalization of *MMP-2*, -9, and *IL-10* mRNA levels. Complementary DNA fragments were amplified by using hydrolysis probes in qPCR cycles for pre-incubation at 95 °C for 10 min, 45 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 30 s and extension at 72 °C for 1 s, and cooling at 40 °C for 30 s. Samples without cDNA templates were utilized as negative controls in each run. Ct values of 40 and above were not included in the study. mRNA levels were detected by the $2^{-\Delta Ct}$ method. Experiments were conducted in duplicate.

Lowry method and enzyme-linked immunosorbent assay (ELISA) test

The Lowry Method was utilized to determine the amount of protein per well in MMP-2 and -9 [18]. Protein levels were measured in adipose tissue homogenates for MMP-2 (SEA100Hu, Cloud-Clone Corp., US) and MMP-9 (SEA553Hu, Cloud-Clone Corp., US) and in plasma for IL-10 (eBioscience Human IL-10 Platinum ELISA, BMS215/2) using the ELISA method. Double standards were run and color intensity was measured in all samples at 450 nm in a microplate reader (BioTek Instruments ELx800).

Statistics

The numerical values were demonstrated as mean ± standard deviation (mean ± SD). The parametric values were indicated by the number of persons (n) and/or as percentages (%). The patient and control groups were evaluated by the Student's t-test anthropometric and biochemical parameters. For the comparison of protein concentrations between patients and controls, the distribution of the normality of continuous variables was checked by Kolmogorov-Smirnov normality testing. Based on the normality testing results, a non-parametric Mann-Whitney U test was conducted for the comparison of protein levels. Statistical evaluation of MMP-2, -9, and IL-10 gene expression was also performed by Mann-Whitney U test. The evaluation of MMP-2, MMP-9, and IL-10 expressions in obesity group and control subjects were determined by both the Pearson's correlation test and non-parametric Spearman's correlation test. SPSS Version 21.0 software program was performed for all statistical analysis (IBM SPSS Inc., Armonk, NY, USA).

Results

Demographic data

The cases participating in the study were assessed for both demographic and biochemical parameters. It was found that weight, height, BMI, fasting blood glucose, fasting insulin, HOMA-IR, LDL-cholesterol, total cholesterol, triglycerides, and HbA_{1c} levels increased significantly in patients with obesity than in controls ($p<0.05$). HDL-cholesterol, systolic, and diastolic blood pressure levels were not statistically significant between patients and controls ($p>0.05$). Four (11.8%) patients were diagnosed with type 2 diabetes and 5 (14.7%) patients were diagnosed with hypertension ($p>0.05$) (Table 1).

MMP-2, MMP-9, and IL-10 mRNA levels and protein concentrations

MMP-2 and *MMP-9* mRNA levels in the adipose tissues were significantly decreased in the obesity group when compared with controls ($p=0.004$, $p=0.045$). However, *IL-10* mRNA levels in the adipose tissues of obesity patients were upregulated when compared with controls ($p=0.010$) (Figure 1A–C).

Protein concentrations of MMP-2 and MMP-9 in adipose tissue homogenates and plasma cytokine levels of IL-10 were also analyzed in patient and control groups. Kolmogorov-Smirnov normality testing and Mann-Whitney U test was conducted for the comparison of MMP-2, MMP-9, and IL-10 protein levels in obesity patients and controls.

While no significant difference was observed in the plasma IL-10 protein levels between obesity patients and controls ($p=0.76$), adipose tissue MMP-2 protein levels significantly diminished ($p=0.001$) and tissue MMP-9 protein levels significantly augmented ($p=0.003$) in the obesity group compared to healthy individuals (Table 2).

Correlation of MMP-2, MMP-9, and IL-10 levels with demographic and clinical parameters

MMP-2, MMP-9, and IL-10 expressions in obesity group and control subjects were investigated by the Pearson's

Table 1: Anthropometric and biochemical parameters in obesity patients and controls.

Parameter	Control (n=36)	Obese (n=34)	p-Value
Age, Year	41.97 ± 16.61	39.08 ± 11.45	0.399
Gender (M/F), n (%)	21/15 (%58.3/%41.7)	10/24 (%29.4/%70.6)	0.015
Weight, kg	66.61 ± 11.05	110.26 ± 21.81	<0.001
Height, cm	174.55 ± 11.27	164.52 ± 8.14	<0.001
BMI, kg/m^2	21.70 ± 1.94	40.80 ± 7.89	<0.001
Fasting glucose, mg/dL	94.11 ± 10.64	111.61 ± 27.20	0.001
Fasting insulin, mg/dL	7.92 ± 2.74	15.12 ± 7.98	<0.001
HOMA-IR	1.85 ± 0.73	4.16 ± 2.42	<0.001
LDL cholesterol, mg/dL	99.47 ± 28.43	138.50 ± 52.58	<0.001
HDL cholesterol, mg/dL	44.94 ± 10.56	47.50 ± 10.41	0.312
Total cholesterol, mg/dL	146.83 ± 44.93	211.05 ± 46.72	<0.001
Triglycerides, mg/dL	85.30 ± 34.09	148.14 ± 84.33	<0.001
SBP, mmHg	129.63 ± 21.97	140.35 ± 23.64	0.053
DBP, mmHg	78.86 ± 11.96	79.79 ± 15.24	0.776
HbA_{1c}	5.14 ± 0.50	6.34 ± 2.10	0.01
T2D (+/–), %	0/36 (0.0/100.0%)	4/30 (11.8/88.2%)	>0.05
Hypertension (+/–), %	1/35 (2.8/97.2%)	5/29 (14.7/85.3%)	>0.05

Data were analyzed with Student's t-test and χ^2 test and are presented as means ± standard deviation (SD). BMI, body mass index; HOMA-IR, insulin resistance; LDL-cholesterol, low density lipoprotein-cholesterol; HDL-cholesterol, high density lipoprotein-cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; T2D, type 2 diabetes.

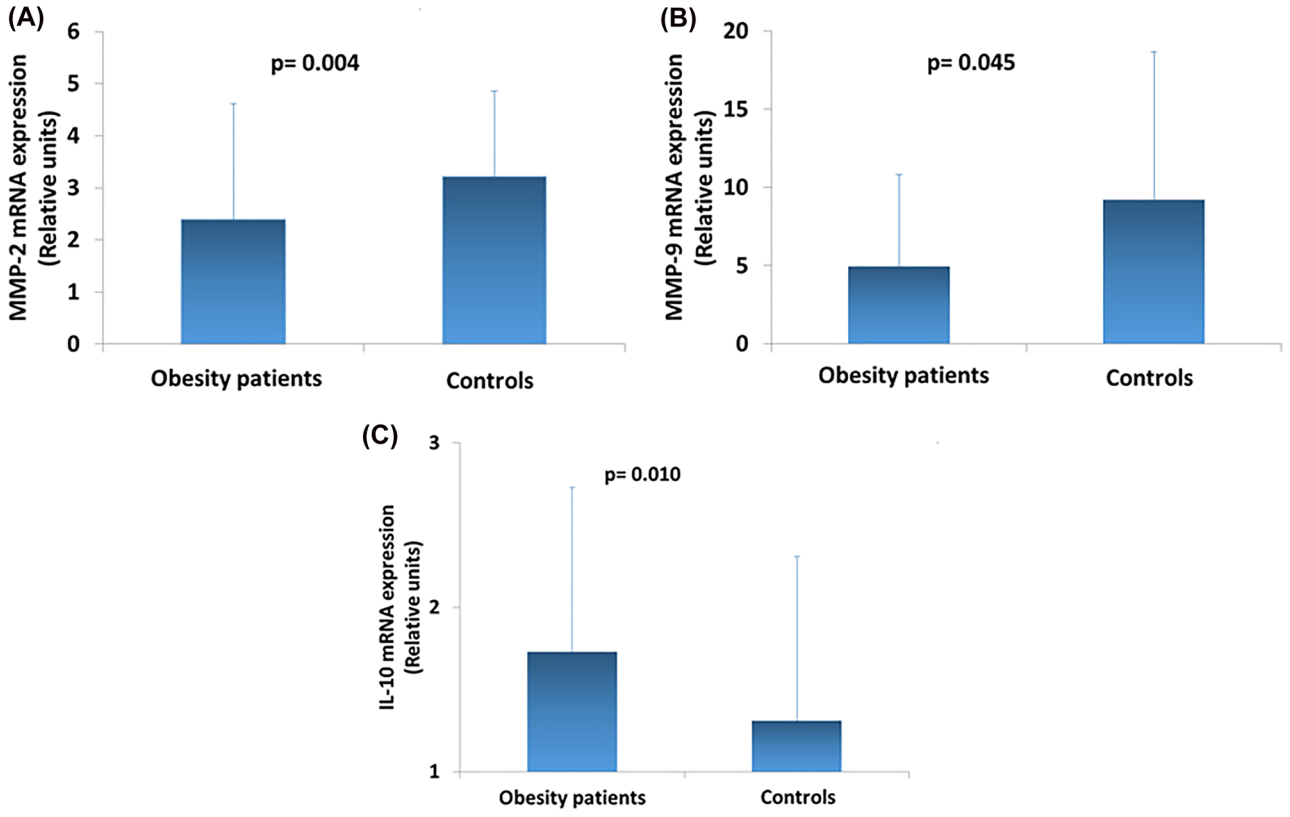


Figure 1: Relative mRNA levels of MMP-2 (A), MMP-9 (B) and IL-10 (C) in patients with obesity and controls.

Table 2: Comparison of MMP-2, MMP-9 and IL-10 protein levels obese patients and controls.

Protein levels	Control (n=36) Median (min-max)	Obese (n=34) Median (min-max)	p-Value
MMP-2, ng/μg protein	0.041 (0.0002–0.1274)	0.023 (0.0052–0.0671)	0.001
MMP-9, ng/μg protein	0.051 (0.0036–1.3195)	0.109 (0.0178–0.6845)	0.003
IL-10, pg/ml	15.683 (1.645–142.65)	12.80 (5.1504–28.3054)	0.76

Values were given as median (min-max). Non-parametric Mann-Whitney U test was performed. MMP-2, matrix metalloproteinase-2; MMP-9, matrix metalloproteinase-9; IL-10, interleukin-10.

correlation test and Spearman's correlation test (Table 3). MMP-2 protein levels got inverse correlation with the weight (r=−0.381, p=0.034) in obesity patients. Furthermore, BMI was also negatively correlated with MMP-2 protein levels (r=−0.411, p=0.022) in the obesity group (Figure 2A). However, a positive correlation was observed between *MMP-2* gene expression and fasting blood glucose levels in patients with obesity (r=0.426, p=0.013) (Figure 2B). Additionally, a significant correlation was seen between insulin resistance (HOMA-IR) and IL-10 protein levels (r=−0.339,

Table 3: The correlation between MMP-2, MMP-9, IL-10 expressions and anthropometric and biochemical characteristics of obesity patients and controls.

Obesity patients	MMP-2 mRNA levels	MMP-9 mRNA levels	IL-10 mRNA levels	MMP-2 protein expression	MMP-9 protein expression	IL-10 protein expression
Age, years	0.16[a]	0.16[a]	−0.06[a]	0.48[a,c]	0.036[a]	0.20[a]
Weight, kg	−0.10[a]	−0.29[a]	−0.23[a]	−0.38[a,b]	0.16[a]	−0.16[a]
Height, cm	0.024	0.042	0.001	0.157	−0.199	−0.117
BMI, kg/m^2	−0.176	−0.263	−0.054	−0.411[b]	0.313	−0.151
Fasting glucose, mg/dL	0.426[b]	0.265	0.262	0.107	−0.185	−0.136
Fasting insulin, mg/dL	−0.146	−0.278	−0.034	−0.115	0.195	−0.259
HOMA-IR	0.009	−0.219	−0.081	−0.015	0.159	−0.339[b]
LDL-cholesterol, mg/dL	−0.107	−0.302	0.027	0.062	0.13	−0.043
HDL-cholesterol, mg/dL	0.158	−0.073	−0.23	0.383[b]	0.004	−0.153
Total cholesterol, mg/dL	−0.063	−0.115	−0.323	0.239	−0.176	−0.034
Triglycerides, mg/dL	0.145	0.022	0.174	0.018	0.132	0.114
SBP, mmHg	−0.074	−0.198	−0.28	0.01	0.033	−0.172
DBP, mmHg	0.01	−0.096	0.025	−0.026	0.105	−0.153
Controls						
Age, years	0.08[a]	−0.06[a]	−0.13[a]	0.33[a]	0.30[a]	−0.12[a]
Weight, kg	0.12[a]	0.12[a]	0.17[a]	0.16[a]	−0.09[a]	−0.21[a]
Height, cm	0.057	0.22	0.158	−0.108	−0.045	−0.358[b]
BMI, kg/m^2	−0.025	0.034	−0.035	0.121	−0.072	−0.145
Fasting glucose, mg/dL	−0.142	−0.10	0.204	−0.021	−0.19	−0.206
Fasting insulin, mg/dL	0.149	0.298	0.212	−0.122	−0.245	−0.054
HOMA-IR	0.085	0.179	0.173	−0.128	−0.3	−0.14
LDL-cholesterol, mg/dL	0.041	0.065	−0.159	−0.135	0.102	−0.067
HDL-cholesterol, mg/dL	0.388[b]	0.026	−0.057	0.269	0.425[b]	−0.208
Total cholesterol, mg/dL	−0.037	0.292	0.031	−0.298	−0.006	0.067
Triglycerides, mg/dL	−0.208	0.251	0.229	−0.114	−0.209	−0.024
SBP, mmHg	−0.117	−0.071	0.017	−0.093	0.092	−0.11
DBP, mmHg	−0.007	0.233	0.051	−0.055	0.111	0.223

Spearman's correlation test has been performed. [a]Pearson's correlation test has been performed. r-Values are given in the Table. [b]$p \leq 0.05$, [c]$p < 0.01$.

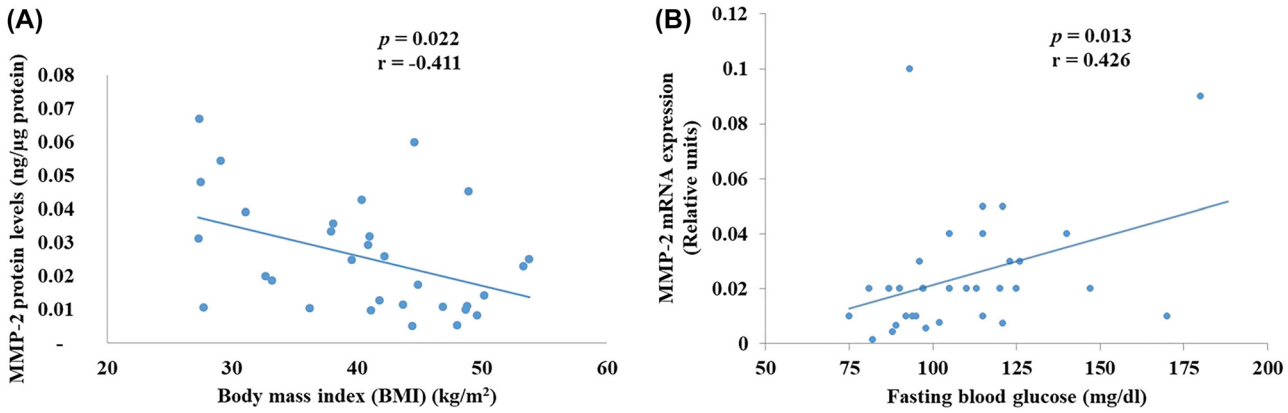


Figure 2: Correlation of adipose tissue MMP-2 protein levels with BMI (A) and MMP-2 mRNA expression with fasting blood glucose levels (B) in patients with obesity.

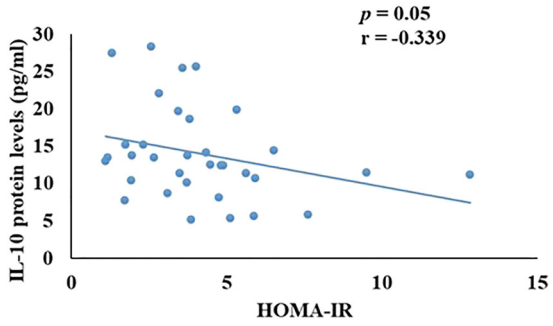


Figure 3: Correlation of serum IL-10 levels with HOMA-IR in patients with obesity.

p=0.05) (Figure 3). However, no significant correlation was seen between HOMA-IR and MMP-2 protein levels (r=–0.015, p=0.93), MMP-9 protein levels (r=0.159, p=0.40).

Discussion

Obesity is a chronic inflammatory disease connected with insulin resistance, hypertension, and dyslipidemia, developed especially as a result of an extreme gathering of fat in the body. Obesity contributes to high morbidity and mortality rates. More than one billion adults worldwide have been reported as overweight [19].

In the study of Dubois et al., where the relation between type 2 diabetes and MMP-2 expression in adipocytes was investigated, MMP-2 was found to be decreased in the obesity group according to controls [20]. In our study, 34 patients with obesity and 36 healthy controls were included. Transcriptional expression of *MMP-2* and *MMP-9* were downregulated in the obesity group compared to controls. MMP-2 protein concentration was also decreased in the obesity group as in controls. We had parallel findings with the study of Dubois et al. [20].

In a study carried out in patients with obesity, having obesity-hypertension and healthy control children and adolescents, MMP-2 levels were augmented in individuals with obesity than in controls [21]. Another study investigated the plasma concentrations of MMP-9, TIMP-1, MMP-8, and myeloperoxidase (MPO) in people with obesity and lean women and concluded that women having obesity demonstrated increased MMP-9 levels and MMP-9:TIMP-1 ratios when compared with lean women [22]. However, Kosmala et al. reported decreased plasma MMP-2 levels in individuals with obesity than in controls [23]. Our study also revealed the diminished adipose tissue MMP-2 levels in obesity patients than in controls consistent with the results of Kosmala et al. Our findings highlight the emphasis of MMP-2 in the advancement of adiposity.

In a study conducted on a total of 170 individuals with obesity, the connection between *MMP-9* expression in adipose tissues and the risk of obesity was examined, and reported that *MMP-9* mRNA expression augmented in the adipose tissues of patients with obesity compared with healthy controls [24]. Nair et al. examined MMP-9 expression in the adipose tissue biopsy specimens of patients with obesity and people having no obesity problem in the Pima Indian population. They reported that MMP-9 expression expanded in obesity patients [25]. Another study reported MMP-9 levels and activity in the adipose tissues of men without diabetes. It was examined that, MMP-9 expression was positively correlated with BMI and negatively correlated with insulin sensitivity. They also observed, MMP-9 was elevated in insulin resistance and was diminished by pioglitazone after treatment which belongs to the thiazolidinedione class with anti-diabetic properties [26]. In a recent study, MMP-9 levels were upregulated in metabolically

unhealthy patients with obesity in the visceral white adipose tissue [27]. We also explored *MMP-9* mRNA levels in the adipose tissues of obesity patients, and we marked that *MMP-9* mRNA levels were lower in obesity patients compared to controls. However, the protein concentration of MMP-9 in the adipose tissues was significantly augmented in obesity patients compared to controls. Our results were consistent with the previous findings in terms of tissue MMP-9 protein expression. The different aspects of the expression patterns between mRNA and protein levels in our study, may arise from the post-transcriptional and post-translational modification mechanisms including RNA processing, RNA stability, protein degradation, and stability and protein modifications. And also, allelic heterogeneity may affect transcriptional expression. Besides, the different results of MMP expression levels in obesity patients in studies may be due to the medications used, the individual's clinical condition, and perhaps ethnic differences.

Interleukin-10 is an anti-inflammatory cytokine secreted from activated macrophages and lymphocytes. Short formation of IL-10 was related to obesity, type 2 diabetes, and metabolic syndrome. In a study conducted on childhood obesity, IL-10 levels were diminished in patients than in controls and had a role in inflammation, tissue-damaging, and obesity [28]. Another study revealed IL-10 expression was four times lower in visceral adipose tissue than subcutaneous adipose tissue, and also was negatively correlated with insulin resistance. It was concluded that IL-10 had a protective role in inflammation [29]. Besides, Pereira et al. reported, *IL-10* gene expression was upregulated in the fat tissue of very severely obesity patients compared to controls. In the same study, IL-10 protein expression was higher in patients with obesity [30]. In our study, *IL-10* gene expression was significantly elevated in the adipose tissues of the obesity group than in healthy controls agreeing with the results of Pereira et al. [30] and additionally, in our study, consistent with previous studies, a significant negative correlation was found between IL-10 levels and insulin resistance.

In a study conducted in 117 obesity patients and 83 healthy controls, IL-10 levels were significantly augmented in the sera of the obesity group [31]. Another study also revealed higher IL-10 serum levels in patients with morbid obesity than in controls. When the same patients underwent bariatric surgery, IL-10 levels were observed to be decreased [32]. In our study, blood specimens were taken before the bariatric surgery and serum IL-10 protein levels were lower in the obesity group than in controls but not significantly altered between the groups.

In this study, MMP-2, -9, IL-10 expressions and anthropometric, biochemical parameters were also examined with both Pierson's correlation test and Spearman's correlation test. There was a positive correlation between *MMP-2* gene expression and fasting blood glucose levels in patients with obesity. In addition, there was a significant negative correlation between MMP-2 protein levels and weight and BMI. Besides, an inverse correlation was examined between *MMP-9* gene expression and fasting insulin levels. In a study by Waters et al., it was reported that there was an inverse correlation between IL-10 and BMI [33]. We also observed a negative correlation between serum IL-10 protein levels and BMI in the obesity group agreeing with the findings of Waters et al. [33]. Nevertheless, this result did not reach statistical significance.

MMP activity may affect the infiltration of immune cells into the adipose tissue and may lead to structural alterations in the remodeling of the ECM, leading to further enlargement of the fat tissue in obesity patients. The increase in MMP activity is a sign of tissue regeneration as well as matrix degradation, suggesting that different combinations of genes and pathways may contribute to the molecular mechanism of obesity. It is thought that the inflammatory response caused by cytokines in the fat tissue in obesity patients may contribute to the formation of metabolic syndrome, which is known to be associated with pro-inflammatory conditions. Thus, the existence of high levels of inflammatory molecules, such as CRP and various cytokines, results in a significant reduction in anti-inflammatory molecules. MMPs may be co-expressed or co-suppressed in response to inflammatory cytokines and growth factors. In our study, the anti-inflammatory cytokine, IL-10, may have suppressed MMP-2.

Our study has some limitations including the lack of the circulating levels of MMP-2, and MMP-9 levels and their correlation with the adipose tissue expression levels. Besides TIMP levels were not detected in our study. The small sample size was another limitation factor in our study. Any relation between MMP-2, MMP-9, and IL-10 expression and adipocyte size or macrophage numbers in the adipose tissues were not emphasized as well.

Conclusions

We suggest that MMP-2, MMP-9, and IL-10 can play a role in obesity and its related phenotypes. Especially MMP-2 may have a significant role in adiposity since both transcriptionally and translationally downregulated in the subcutaneous adipose tissues of obesity patients. Fasting blood glucose levels may be related to the transcriptional

expression of *MMP-2* in obesity. New studies should be conducted on *MMP-2* and *-9* and *IL-10* genes in a large number of patients and controls both in tissues and sera to contribute to an improved understanding of the therapeutic aspects of obesity and obesity-related diseases in the future.

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Competing interests: Authors state no conflict of interest.
Informed consent: Informed consent was obtained from all individuals included in this study.
Ethical approval: This investigation was performed according to the Declaration of Helsinki and was agreed upon by the local ethics committee of Cerrahpasa Medical Faculty (Ethics Committee issue #40791).

References

1. Barlow SE, Dietz WH. Obesity evaluation and treatment: expert committee recommendations. Pediatrics 1998;102:e29.
2. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 2004;89:2548–56.
3. Das UN. Is obesity an inflammatory condition? Nutrition 2001;17: 953–66.
4. Chavey C, Mari B, Monthouel M-N, Bonnafous S, Anglard P, Van Obberghen E, et al. Matrix metalloproteinases are differentially expressed in adipose tissue during obesity and modulate adipocyte differentiation. J Biol Chem 2003;278: 11888–96.
5. Nagase H, Visse R, Murphy G. Structure and function of matrix metalloproteinases and TIMPs. Cardiovasc Res 2006;69:562–73.
6. Lagente V, Boichot E. Matrix metalloproteinases in tissue remodelling and inflammation. Berlin: Springer Science & Business Media; 2008.
7. Miksztowicz V, Siseles N, Machulsky NF, Schreier L, Berg G. Increase in MMP-2 activity in overweight and obese women is associated with menopausal status. Climacteric 2012;15: 602–6.
8. Bouloumié A, Sengenes C, Portolan G, Galitzky J, Lafontan M. Adipocyte produces matrix metalloproteinases 2 and 9: involvement in adipose differentiation. Diabetes 2001;50:2080–6.
9. Halade GV, Jin Y-F, Lindsey ML. Matrix metalloproteinase (MMP)-9: a proximal biomarker for cardiac remodeling and a distal biomarker for inflammation. Pharmacol Ther 2013;139: 32–40.
10. Shin YH, Kim KE, Lee Y-J, Nam J-H, Hong YM, Shin H-J. Associations of matrix metalloproteinase (MMP)-8, MMP-9, and their inhibitor, tissue inhibitor of metalloproteinase-1, with obesity-related biomarkers in apparently healthy adolescent boys. Korean J Pediatr 2014;57:526.
11. Varma MC, Kusminski CM, Azharian S, Gilardini L, Kumar S, Invitti C, et al. Metabolic endotoxaemia in childhood obesity. BMC Obes 2015;3:1–8.
12. Woessner JF, Jr. Matrix metalloproteinases and their inhibitors in connective tissue remodeling. Faseb J 1991;5:2145–54.
13. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol 2011;11:85–97.
14. Sivasubramanian N, Coker ML, Kurrelmeyer KM, MacLellan WR, DeMayo FJ, Spinale FG, et al. Left ventricular remodeling in transgenic mice with cardiac restricted overexpression of tumor necrosis factor. Circulation 2001;104:826–31.
15. Kawamura N, Kubota T, Kawano S, Monden Y, Feldman AM, Tsutsui H, et al. Blockade of NF-kappaB improves cardiac function and survival without affecting inflammation in TNF-alpha-induced cardiomyopathy. Cardiovasc Res 2005;66:520–9.
16. Stearns ME, Kim G, Garcia F, Wang M. Interleukin-10 induced activating transcription factor 3 transcriptional suppression of matrix metalloproteinase-2 gene expression in human prostate CPTX-1532 Cells. Mol Cancer Res 2004;2:403–16.
17. Holven KB, Halvorsen B, Bjerkeli V, Damås JK, Retterstøl K, Mørkrid L, et al. Impaired inhibitory effect of interleukin-10 on the balance between matrix metalloproteinase-9 and its inhibitor in mononuclear cells from hyperhomocysteinemic subjects. Stroke 2006;37:1731–6.
18. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193:265–75.
19. Chiang DJ, Pritchard MT, Nagy LE. Obesity, diabetes mellitus, and liver fibrosis. Am J Physiol Liver Physiol 2011;300:G697–702.
20. Dubois SG, Tchoukalova YD, Heilbronn LK, Albu JB, Kelley DE, Smith SR, et al. Potential role of increased matrix metalloproteinase-2 (MMP2) transcription in impaired adipogenesis in type 2 diabetes mellitus. Biochem Biophys Res Commun 2008;367:725–8.
21. Belo VA, Lacchini R, Miranda JA, Lanna CMM, Souza-Costa DC, Tanus-Santos JE. Increased activity of MMP-2 in hypertensive obese children is associated with hypoadiponectinemia. Obesity 2015;23:177–82.
22. Andrade VL, Petruceli E, Belo VA, Andrade-Fernandes CM, Russi CVC, Bosco AA, et al. Evaluation of plasmatic MMP-8, MMP-9, TIMP-1 and MPO levels in obese and lean women. Clin Biochem 2012;45:412–5.
23. Kosmala W, Plaksej R, Przewlocka-Kosmala M, Kuliczkowska-Plaksej J, Bednarek-Tupikowska G, Mazurek W. Matrix metalloproteinases 2 and 9 and their tissue inhibitors 1 and 2 in premenopausal obese women: relationship to cardiac function. Int J Obes 2008;32:763–71.
24. Das SK, Ma L, Sharma NK. Adipose tissue gene expression and metabolic health of obese adults. Int J Obes 2015;39:869–73.

25. Nair S, Lee YH, Rousseau E, Cam M, Tataranni PA, Baier LJ, et al. Increased expression of inflammation-related genes in cultured preadipocytes/stromal vascular cells from obese compared with non-obese Pima Indians. Diabetologia 2005;48:1784–8.
26. Unal R, Yao-Borengasser A, Varma V, Rasouli N, Labbate C, Kern PA, et al. Matrix metalloproteinase-9 is increased in obese subjects and decreases in response to pioglitazone. J Clin Endocrinol Metab 2010;95:2993–3001.
27. Doulamis IP, Konstantopoulos P, Tzani A, Antoranz A, Minia A, Daskalopoulou A, et al. Visceral white adipose tissue and serum proteomic alternations in metabolically healthy obese patients undergoing bariatric surgery. Cytokine 2019;115: 76–83.
28. Arslan N, Erdur B, Aydin A. Hormones and cytokines in childhood obesity. Indian Pediatr 2010;47:829–39.
29. McLaughlin T, Liu L-F, Lamendola C, Shen L, Morton J, Rivas H, et al. T-cell profile in adipose tissue is associated with insulin resistance and systemic inflammation in humans. Arterioscler Thromb Vasc Biol 2014;34:2637–43.
30. Pereira S, Teixeira L, Aguilar E, Oliveira M, Savassi-Rocha A, Pelaez JN, et al. Modulation of adipose tissue inflammation by FOXP3+ Treg cells, IL-10, and TGF-β in metabolically healthy class III obese individuals. Nutrition 2014;30:784–90.
31. Schmidt FM, Weschenfelder J, Sander C, Minkwitz J, Thormann J, Chittka T, et al. Inflammatory cytokines in general and central obesity and modulating effects of physical activity. PLoS One 2015;10:e0121971.
32. Arismendi E, Rivas E, Agustí A, Ríos J, Barreiro E, Vidal J, et al. The systemic inflammome of severe obesity before and after bariatric surgery. PLoS One 2014;9:e107859.
33. Waters KA, Mast BT, Vella S, De la Eva R, O'Brien LM, Bailey S, et al. Structural equation modeling of sleep apnea, inflammation, and metabolic dysfunction in children. J Sleep Res 2007;16: 388–95.