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ORIGINAL ARTICLE

RANK/RANKL/OPG pathway is an important for the epigenetic regulation of obesity

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Abstract

Obesity is a complex disorder that is influenced by genetic and environmental factors. DNA methylation is an epigenetic mechanism that is involved in development of obesity and its metabolic complications. The aim of this study was to investigate the association between the *RANKL* and *c-Fos* gene methylation on obesity with body mass index (BMI), lipid parameters, homeostasis model assessment of insulin resistance (HOMA-IR), plasma leptin, adiponectin and resistin levels. The study included 68 obese and 46 non-obese subjects. Anthropometric parameters, including body weight, body mass index, waist circumference, and waist-hip ratio, were assessed. Serum glucose, triglycerides (TG), total cholesterol, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), plasma leptin, adiponectin and resistin levels were measured. Methylation status of *RANKL* and *c-Fos* gen were evaluated by MS-HRM. Statistically significant differences were observed between obese patients and the controls with respect to *RANKL* and *c-Fos* gene methylation status ($p < 0.001$). Also, statistically significant importance was observed *RANKL* gene methylation and increased level of leptin in obese subjects ($p = 0.0081$). At the same time, statistically significant association between methylation of *c-Fos* and increased level of adiponectin was observed in obese patients ($p = 0.03$) On the other hand, decreased level of resistin was observed where the *c-Fos* was unmetyladed in controls ($p = 0.01$). We conclude that methylation of *RANKL* and *c-Fos* genes have significant influences on obesity and adipokine levels. Based on literature this was the first study which shows the interactions between *RANKL* and *c-Fos* methylation and obesity.

Keywords Obesity · Epigenetics · Methylation · *RANKL* · *c-Fos*

Introduction

Obesity is one of the most important health problems with increasing in epidemic proportions in developed countries [1, 2]. It increases the risk of many associated comorbidities such as diabetes, heart disease, cognitive decline, infertility and certain cancers [3–9]. At the cellular level, inflammation and endoplasmic reticulum (ER) stress was induced by obesity [10] and triggers insulin/leptin resistance, hyperphagia, hyperglycemia and fatty liver disease [11]. Increased level of leptin or decreased level of adiponectin has an affect bone and bone resorption in obesity [12]. Leptin, is a small polypeptide hormone which is secreted by the adipocytes, and supports adipose as an energy storing organ also an active endocrine tissue [13].

The interaction between obesity and chronic inflammatory response, abnormal cytokine production, increased acute-phase reactants, and activation of inflammatory signalling pathways had been shown by the different experimental, epidemiological, and clinical studies [14]. Increased level of TNF-α, leptin and decreased level adiponectin were observed in obese patients and these triggers RANK/RANKL/OPG pathway activation [12, 15]. Researchers identified that increased level of cytokines promotes

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osteoclast activity by activating RANK/RANKL/osteoprotegerin pathway in obesity [12]. RANKL is a member of tumour necrosis factor (TNF) cytokine family and has an important role during the bone reconstruction [16, 17]. RANKL/RANK expressed in different tissues like bone bone marrow, lymphoid tissues [18], the hypothalamus and septal regions of the brain [18–20]. Also, RANKL expressed in the immune system which helps to regulation survival and function of the dendritic cells [21–23]. Obesity is characterized with chronic inflammation and the increased level of circulating and tissue proinflammatory cytokines which promotes osteoclast activity and bone resorption via receptor activator of NF-κB (RANK)/RANK ligand/osteoprotegerin pathway [12]. Thus, RANKL triggers different protein–protein interactions, enzyme–substrate reactions or protein translocation reactions. These reactions can be stimulated by directly RANKL/RANK system, or induced or enhanced in vivo [24]. Due to this direct or indirect catalytic and pleiotropic effects of RANKL in different tissue systems, takes attention of the researchers who works with bone or bone related diseases and obesity.

c-Fos gene encodes a transcription factor that involved in extracellular signal transduction and also important for external stimuli response of neurons. Acute stress decreased Fos- the glucagon-like peptide-1 receptor (GLP1R) expression in the lateral hypothalamic area [25]. Chagra et al. showed that food intake may be changed *c-Fos* expression and play a role in obesity [26].

Numerous studies have reported a significant association between DNA methylation with body weight regulation, adipogenesis and obesity. Many studies showed that DNA methylation at metabolic genes associated with obesity, such as HIF3A and SREBF1 [27, 28]. There is no published work showes the relationship between the *RANKL* and *c-Fos* genes methylation status and obesity. The aim of our study was to investigate the association between *RANKL* and *c-Fos* genes methylation status, BMI, lipid parameters, HOMA-IR, plasma leptin, adiponectin and resistin levels in obese subjects compared to non-obese subjects.

Materials and methods

Subjects

The study included a retrospective investigation of epigenetic alterations in obesity. Participants in this study were patients who attended the outpatient clinic of the Cengiz Topel Governmental Hospital, Yesilyurt. In the first group contained 68 obese patients who have a mean age of 42.43 ± 1 years and BMI of 35.42 ± 5.67 kg/m^2. The second group, control group, included 46 non-obese subjects. The mean age of these subjects was 39.39 ± 1.196 years and their mean BMI was 22.58 ± 2.12 kg/m^2.

We excluded adults with cancer, diabetes mellitus, hypertension, dyslipidemias, liver cirrhosis, kidney lithiasis, thyroid, cardiovascular, or any active inflammatory disease. None of the participants received any medications or applied any dietary or exercise program during the study. Medical history were questioned and written informed consent form obtained from all the subjects. The study protocol was approved by the Research Ethics Committee of the Near East University and performed in accordance with the Declaration of Helsinki (Project No: SAG-2016-2-012).

Anthropometric measurements

Weight (kg), height (m), hip circumference (cm) and waist circumference (cm) were measured at fasting state with light clothes from each subject. Hip circumference was measured by placing a measuring tape around fullest portion of the patient's hips. Waist circumference was measured midway between the lowest rib (laterally) and the iliocristale landmark with flexible tape. BMI was estimated by dividing body weight (kg) by the square of height (m^2). BMI ≥ 30 kg/m^2 was accepted as an obese [29].

Biochemical parameters

Blood samples were obtained after an overnight fast. Circulating levels of serum glucose, triglycerides (TG), total cholesterol, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured using an automated analyzer following an overnight fasting state (Abbott Architect C8000). Insulin concentrations were measured using an electrochemiluminescence assay (Ref. 12017547; Elecsys Corp., Lenexa, KS). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to the formula: fasting insulin (μU/mL) × fasting glucose (mmol/L) divided by 22.5 [30].

Plasma leptin (ng/mL), adiponectin (μg/mL) and resistin (ng/mL) levels were determined by enzyme linked immunosorbent assay (ELISA) kits (DRG Intl., Inc., USA for leptin and Biovendor Laboratory, Inc., Brno, Czech Republic for resistin and adiponectin) according to the manufacturers' protocols.

Determination of C-FOS and RANKL methylation status

Genomic DNA was extracted from whole blood samples according to the Qiagane AllPrep DNA/RNA/Protein isolation kit and NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific) was used to measure quantity of DNA. To determine the *c-Fos* and *RANKL* methylation, first bisulfite modification reaction was applied by using the EpiTect Bisulfite Kit according to the manufacturers' protocol (Qiagen, Manchester, UK). Universal methylated and unmethylated DNA (EpiTect Control DNA Set, Cat No./ID: 59568) were used as methylated and unmethylated controls. We used QIAGEN Rotor Gene Q for MS-HRM to detect the methylation status of our samples (Qiagen, Manchester, UK). Primers were designed according to the EpiTect® HRM™ PCR Handbook (Table 1). The MS-HRM analysis was performed according to EpiTect® HRM™ PCR Handbook protocol. We used comparable amounts of template genomic DNA for all samples resulting in CT values below 30 and differing by no more than 3 CT values.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation (SD). Comparison between groups were analysed using Student's *t* test and the χ^2 test for continuous variables and categorical variables, respectively. Continuous variables in the two subgroups was performed by Mann–Whitney *U* test. A p value of < 0.05 was considered statistical significance. All statistical analyses were performed using the GraphPad Prism 7 software.

Results

The anthropometric and metabolic characteristics of the patients were presented in Table 2. There is a no statistical significance observed between obese and non-obese subjects age. The plasma glucose, total cholesterol, triglycerides, LDL-cholesterol, HOMA-IR, leptin, and resistin levels were significantly higher in obese than non-obese subjects ($p < 0.05$). Additionally, the level of mean HDL-cholesterol and adiponectin were significantly decreased in obese subjects than non-obese subjects ($p < 0.001$).

Table 1 The primer sequences used for the MS-HRM are listed

Gene	Primer sequences
C-FOS	F 5′-TGTAAG GTAGTTTTA TTGATAAAA -3′ R 5′-TTAAAT ATCCCTCAT CCTAAATA-3′
RANKL	F 5′- GGGAGT TAGAGGTGG GAGTG-3′ R 5′-GGCTTT AAATCCTAA AAC-3′

Table 2 Baseline characteristics of studied population

Parameter	Non-obese subjects (n = 46)	Obese subjects (n = 68)	*p*
Age	39.39 ± 1.196	42.43 ± 1	0.05
BMI (kg/m^2)	22.58 ± 0.3	35.42 ± 0.68	<0.001
Waist circumference (cm)	84.89 ± 1.26	113.3 ± 1.58	<0.001
Hip circumference (cm)	99.8 ± 1.05	119.3 ± 1.16	< 0.001
Fasting glucose (mg/dL)	90.91 ± 1.09	103.4 ± 2.4	< 0.001
Total cholesterol (mg/dL)	204.5 ± 4.13	226.4 ± 4.56	< 0.001
LDL-cholesterol (mg/dL)	129.6 ± 4.02	141.8 ± 3.91	0.03
HDL-cholesterol (mg/dL)	57.15 ± 1.70	46.16 ± 1.18	< 0.001
Triglycerides (mg/dL)	98.26 ± 7.67	173.4 ± 9.41	< 0.001
HOMA-IR	2.098 ± 0.12	4.58 ± 0.36	< 0.001
Leptin (ng/mL)	9.44 ± 0.78	23.85 ± 1.6	< 0.001
Adiponectin (μg/mL)	21.47 ± 1.6	9.99 ± 0.6	< 0.001
Resistin (ng/mL)	6.27 ± 0.39	8.96 ± 0.31	< 0.001

Data are expressed as mean ± SD and were compared by *t* test

BMI body mass index, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *HOMA-IR* homeostasis model assessment of insulin resistance

Methylation status of *RANKL* and *c-Fos* genes

Compared statistical analysis of the RANKL gene methylation status in obese and non-obese subjects showed in Table 3. *RANKL* gene methylated in 4 of the 65 obese subjects (20%) and 16 of the 46 non-obese subjects (80%). There was significant difference between methylation and obese and non-obese subjects ($p < 0.001$).

c-Fos gene methylated in 53 of the 65 obese subjects (69.77%) and 23 of the 46 non-obese subjects (30.26%). There was significant difference between methylation and obese and non-obese subjects ($p < 0.001$) (Table 4).

Table 3 Methylation status of the *RANKL* gene in obese and non-obese subjects

Subjects	*RANKL* gene		
	Methylation	Unmethylation	p Value
Obese	20% (4)	73.86% (65)	$p < 0.001$
Non-obese	80% (16)	26.14% (23)	

Table 4 Methylation status of the *c-Fos* gene in obese and non-obese subjects

Subjects	*c-Fos* gene		
	Methylation	Unmethlation	p Value
Obese	69.77% (53)	39.39% (13)	$p < 0.001$
Non-obese	30.26% (23)	60.61% (20)	

Relationship between anthropometric and metabolic characteristics and *RANKL* gene methylation

There is a statistically significant association detected between methylated *RANKL* and leptin level in obese subjects ($p < 0.001$). The level of leptin were significantly higher in the *RANKL* methylated cases ($p = 0.0081$) (Table 5).

Relationship between anthropometric and metabolic characteristics and *c-Fos* gene methylation

Resistin level was significantly higher in unmethylated *c-Fos* in non-obese cases ($p = 0.01$). The level of the resistin is lower in *c-Fos* methylated non-obese cases ($p = 0.01$). Moreover, the level of the adiponectin were significantly higher in *c-Fos* methylated obese cases (Table 6).

Discussion

Genetic factors, unhealthy eating patterns, or a combination of these factors are the major inducers of an obesity [30]. Studies showed that obesity is the major cause of mortality and morbidity [31]. Several researchers show the effects of overweight on bone formation by decreasing apoptosis and effects on osteoporosis in humans [32–35].

To date, leptin (*LEP*), leptin receptor (*LEPR*), proopiomelanocortin (*POMC*), prohormone convertase 1 (*PCSK1*), melanocortin 4 receptor (*MC4R*), single-minded homologue 1 (*SIM1*), brain-derived neurotrophic factor (*BDNF*), and neurotrophic tyrosine kinase receptor type 2 (*NTRK2*) are well-established monogenic obesity related genes and mutations of these genes are related with early onset of obesity [36]. Until now, many genes identified which their expression is regulated with epigenetically and important for the obesity development, metabolic disorders, appetite control, insulin signaling, immunity, inflammation, growth, and circadian clock regulation [37–40]. Genome-wide DNA methylation analysis in leucocytes and adipose tissue shows abnormal methylation pattern in *CLOCK* (clock circadian regulator), *BMAL1* (aryl hydrocarbon receptor nuclear translocator-like), *PER2* (period circadian 2) genes which are known as circadian clock genes and *UBASH3A* (ubiquitin-associated and SH3 domain-containing protein A) and *TRIM3* (tripartite motif containing 3) genes [41–43].

Until now, epigenetic regulation of LEP, ADIPOQ, *PGC1α*, IGF-2, *IRS-1*, *LY86*, *MEST*, *PEG3*, *NNAT*, *PLAGL1*, *MEG3*, *NPY*, *IL6*, *TNF*, *TFAM* and *GLUT4* genes

Table 5 Anthropometric and metabolic characteristics across *RANKL* gene methylation status of obese and non-obese subjects

Parameter	Non-obese subjects			Obese subjects		
	Methylated (n = 16)	Unmethylated (n = 23)	p	Methylated (n = 4)	Unmethylated (n = 65)	p
BMI (kg/m^2)	22.87 ± 1.86	22.4 ± 2.43	0.77	36.69 ± 1.14	35.47 ± 5.76	0.83
Waist circumference (cm)	87.5 ± 8.41	83.87 ± 8.38	0.11	115 ± 10.46	113.1 ± 13.22	0.58
Hip circumference (cm)	102 ± 6.59	98.04 ± 7.74	0.06	119.5 ± 5.06	119.3 ± 9.78	0.78
Fasting glucose (mg/dL)	90.69 ± 6.34	91.61 ± 8.23	0.94	115 ± 30.28	102.7 ± 18.93	0.23
Total cholesterol (mg/dL)	202.3 ± 32.04	205.7 ± 27.29	0.63	212.8 ± 37.87	227.3 ± 37.47	0.62
LDL-cholesterol (mg/dL)	126.1 ± 29.19	130 ± 28.31	0.65	142.8 ± 39.27	142 ± 32.1	0.92
HDL-cholesterol (mg/dL)	57.31 ± 10.64	59.04 ± 12.49	0.63	39.5 ± 7.14	46.55 ± 9.72	0.12
Triglycerides (mg/dL)	99.75 ± 42.67	92.39 ± 52.93	0.24	151 ± 26.62	174.5 ± 79.01	0.53
HOMA-IR	2.17 ± 0.71	2.102 ± 0.89	0.55	5.66 ± 2.92	4.57 ± 3.02	0.45
Leptin (ng/mL)	10.9 ± 4.27	9.22 ± 6.07	0.21	32.09 ± 15.86	20.85 ± 8.19	0.0081
Adiponectin (μg/mL)	17.73 ± 9.18	24.57 ± 11.26	0.10	10.57 ± 2.89	9.91 ± 5.21	0.62
Resistin (ng/mL)	6.79 ± 2.23	6.55 ± 2.99	0.60	8.08 ± 2.17	8.97 ± 2.61	0.66

Data are expressed as mean ± SD. For the comparison of subgroups, analysis of variance followed by Mann–Whitney *U* test was performed

BMI body mass index, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *HOMA-IR* homeostasis model assessment of insulin resistance

Table 6 Anthropometric and metabolic characteristics across *c-Fos* gene methylation status of obese and non-obese subjects

Parameter	Non-obese subjects			Obese subjects		
	Methylated ($n = 23$)	Unmethylated ($n = 20$)	p	Methylated ($n = 53$)	Unmethylated ($n = 13$)	p
BMI (kg/m^2)	22.29 ± 2.32	22.9 ± 1.85	0.55	35.28 ± 4.33	36.28 ± 9.74	0.43
Waist circumference (cm)	83.78 ± 9.54	86.3 ± 7.92	0.34	113.5 ± 12.34	111.5 ± 16.4	0.39
Hip circumference (cm)	99.83 ± 6.04	99.85 ± 8.81	0.63	119.1 ± 8.82	120.2 ± 13.17	0.86
Fasting glucose (mg/dL)	91.48 ± 8.49	91.1 ± 6.30	0.99	104 ± 20.1	96.85 ± 10.79	0.37
Total cholesterol (mg/dL)	199.7 ± 23.58	210.5 ± 32.21	0.36	227.7 ± 37.38	227.6 ± 38.53	0.90
LDL-cholesterol (mg/dL)	126.9 ± 24.19	133 ± 31.99	0.61	141.9 ± 32.33	145.7 ± 32.91	0.77
HDL-cholesterol (mg/dL)	56 ± 11.89	58.4 ± 11.44	0.88	45.74 ± 9.72	49.54 ± 9.10	0.14
Triglycerides (mg/dL)	110.4 ± 63.22	87.75 ± 37.07	0.38	176.6 ± 84.71	161.8 ± 47.67	0.99
HOMA-IR	2.10 ± 0.94	2.12 ± 0.71	0.58	4.85 ± 3.2	3.46 ± 1.67	0.18
Leptin (ng/mL)	8.93 ± 5.06	10.01 ± 5.86	0.62	24.32 ± 12.52	23.88 ± 16.23	0.59
Adiponectin (μg/mL)	22.73 ± 11.73	19.35 ± 8.42	0.43	10.39 ± 5.19	7.98 ± 4.79	0.03
Resistin (ng/mL)	5.47 ± 2.47	7 ± 2.89	0.01	8.98 ± 2.69	8.97 ± 2.22	0.89

Data are expressed as mean ± SD. For the comparison of subgroups, analysis of variance followed by Mann–Whitney *U* test was performed

BMI body mass index, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *HOMA-IR* homeostasis model assessment of insulin resistance

had been reported related with obesity or weight loss [44]. Also, obesity causes activation of the c-Jun N-terminal kinase (JNK) and nuclear factor-kappa B (NF-κB) signaling pathways [45]. While receptor activator of NF-κB (RANKL) binds to its receptor (RANK) and activates the NF-κB pathway and activation of the pathway triggers pro-inflammatory cytokines expression [46]. The expression of RANK and RANKL are related with glycemic control and obesity and these genes are expressed in human liver tissue and pancreatic β-cells [47]. Kiechl and colleagues showed that the concentration of soluble RANKL was associated with insulin resistance [47]. As we mentioned previously, activation of the transcription factor nuclear factor-κB (NF-κB) triggers the activation of inflammatory signaling pathways and related with insulin resistance and β-cell dysfunction [48, 49]. It activates T cells and endothelial cells or adipocytes [47] but there is a unknown interaction between skeleton and the immune systems which may contribute to hepatic insulin resistance. RANKL could be used to connect interaction between immune activation, bone resorption and obesity [47]. In our work, we identified statistically significant RANKL unmethylated in obese group (73.86%) and 80% of the non-obese group were methylated ($p < 0.001$). This confirms Kiechl and colleagues works [47] but due to the retrospective nature of our study we cannot confirm gene expression level and based on current literature this was the first study which shows the interaction epigenetic regulation of RANKL and obesity. This result should be led light to the further epigenetic studies.

Zhu and colleagues shows in vitro treatment in mice and lacked RANKL in their daily food. They identified changes in *c-Fos* expression during the response of peripheral RANKL in hypothalamus and they conclude that RANKL plays an important role as a food inhibitor and causes decreased body weight of mice [50]. Ostrowska et al. identified increased level of RANKL circulation in patients with anorexia nervosa, and then showed the RANKL level was depended on the severity of the anorexia nervosa [51, 52]. In in vitro studies shows injection of adenovirus vector harbouring murine soluble *RANKL* cDNA in mice triggers exhibit reduced food intake and body weight [53]. Zampetti and colleagues analysed OPG/RANKL ratio and they showed higher level of OPG/RANKL were associated with overweight/obese children and adolescents [54]. Studies showed that RANKL regulates hepatic insulin sensitivity and blockage of RANKL signalling proves insulin sensitivity and normalizes glucose concentrations in hepatocytes [55, 56]. On the other hand, Yeşilkaya and colleagues analysed OPG and RANKL levels in obese children but they did not find significant differences between obese and non-obese children [57]. In this work, we identified statistically significant differences of RANKL methylation between obese and non- obese cases. *RANKL* gene were unmethylated in obese group (73.86%) and 80% of the non-obese group were methylated ($p < 0.001$). Based on current literature this was the first study which shows the interaction epigenetic regulation of RANKL and obesity.

Within the hypothalamic regions, neurons expressed feeding-related markers. Researchers identified strong interaction between neuronal activation and c-Fos expression. It was triggers AgRP, MC4R and GLP1R expression in neurons [58]. Acute stress decreased Fos-GLP1R expression in the lateral hypothalamic area and increased orexigenic signaling in the brain [59]. Luna-Illades et al. showed that obesity diminished *Fos* expression in hypothalamic nuclei of obese *N. Alstoni* mice [60]. Considering our results which showed that *c-Fos* gene were methylated in 69.77% of the cases in obese group and in non-obese group 60.61% were unmethylated, one could then speculate that *c-Fos* gene methylation can be regarded as a susceptibility to obesity.

The role of estrogene on bone acts via RANKL and OPG and the deficiency of estrogen during the postmenopausal women causes increased RANKL level, and triggers osteoclastogenesis. Also, the bone protection of obese individuals who have a high level of leptin will be defective. Leptin plays an important role during the regulation of weight, energy expenditure, bone metabolism [61]. Leptin and receptors had been widely studied, and results show that leptin plays a role for function of metabolic functions, neuroendocrine function, immune function, reproduction, and bone metabolism [62–65]. Also, the concentration of leptin reflects energy storage in adipose tissue, and circulating leptin level was related with the amount of body fat [61]. Leptin is an adipocytokine produced in white adipose tissue and plays important roles in obesity, food intake, glucose homeostasis, and energy expenditure. It may participate in several mechanisms of obesity associated disease such as hypertension, metabolic syndrome, cardiovascular disease and bone diseases [46, 62]. Receptor activator of nuclear factor-kappaB ligand (RANKL) and its receptor (RANK) have been described for their roles in the regulation of bone resorption. Leptin induces synaptic activity that signals to promotes osteoblast proliferation and suppresses bone resorption effects of osteoclasts via RANKL synthesis [66]. Elefteriou et al. showed that leptin could inhibit the expression of RANKL in osteoblasts and therefore supressed osteoclast differentiation [67]. Moreover, Holloway et al. reported that leptin can osteoclast generation in vitro by decreasing the receptor activator of RANKL in stromal cells [68]. Consistent with these previous studies, we showed that RANKL methylated cases had significantly higher serum leptin levels than unmethylated RANKL gene cases in obese group. It can be concluded that leptin may regulate bone metabolism through RANKL synthesis.

Overall, our data support the conclusion that RANKL has a important role in the pathogenesis of obesity and provides a link between serum leptin level. These findings hold promise for the future development of new therapeutic and preventive approaches.

Adiponectin is one of the key adipokine which is secreted in adipocytes of adipose tissue and has an important role during the carbohydrate regulation and fat metabolism in insulin-sensitive tissues, and acts as an endogenous insulin-sensitizer [69]. Adiponectin level decrease in obese individual and inversely correlated with the presence of obesity-related complications [70–72]. On the other hand, resistin is another peptide hormone with biological properties opposite to adiponectin. It was found many tissues but it is expressed mainly in the adipose tissue [73]. In humans, obesity was found to be associated with high resistin serum levels [74, 75]. Hirai et al. discovered that resistin increased c-Fos transcription factor expression, however adiponectin suppressed resistin induced *c-Fos* expression in the intracellular signalling pathway [76]. These data suggest that adiponectin and resistin may show opposite effects on metabolism via different expression levels of transcription factors such as c-Fos. Considering our results, resistin level was significantly higher while the *c-Fos* was unmethylated in non-obese group. Furthermore, higher serum adiponectin level was observed when the *c-Fos* was methylated in obese group. Thus, our results may suggest that methylation status of *c-Fos* gene may be related with different levels and metabolic effects of resistin and adiponectin in obesity.

As a consequence of the increased power of this study is that the first study which showed association of *RANKL* and *c-Fos* gene methylation with anthropometric parameters, lipid profile, HOMA-IR, leptin, adiponectin and resistin levels. Also, it was performed in well characterized individuals, with or without obesity. These results suggest that epigenetic studies are another perspective for the identification of the potential genes that play a role in obesity and weight regulation. Further insights into the underlying biological mechanisms and different pathways will be needed for the development effective treatment strategies and management of these traits.

In conclusion, our results suggest that the *RANKL* and *c-Fos* gene methylation status have association with obesity. Additionally, they have significant role on leptin, resistin and adiponectin levels. Further assessment of all other possible methylation status of different genes which might affect obesity and adipokine levels is required. Nowadays, the increased number of studies on obesity is even higher, because of increased prevalence worldwide, causes of many other pathologies like; alterations of reproductive capacity and epigenetic changes. The identification of epigenetic alterations of obesity is important for disease outcome and development of most effective therapy. The reversible nature of epigenetic modifications makes them important targets for a possible epigenetic therapy targets in obesity.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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