Association between the circulating leptin levels and the biomarkers of oxidative stress and inflammation among Iranian overweight and obese adults

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Abstract

Background: Oxidative stress in obese people is an important pathogenic mechanism of an obesity-associated metabolic syndrome. We evaluated the association between circulating leptin levels with biomarkers of oxidative stress in overweight and obese participants.

Methods: This study was performed on 189 overweight and obese people aged 18-60 years old. Serum leptin, superoxide dismutase (SOD), high-sensitivity C-reactive protein (hs-CRP), homocysteine (Hcy), thiobarbituric acid reactive substances (TBARS) and amyloid A (SAA) concentrations were measured. Pearson correlation and multiple linear regressions were used to assess the relationships.

Results: We found that among the biomarkers of oxidative stress and inflammation in participants, serum hs-CRP and SAA were positively correlated with BMI ($\beta=0.003$, $P<0.001$ and $\beta=7.83$, $P<0.001$, respectively). This relationship remained significant even after controlling other oxidative stress and inflammatory indicators ($\beta=0.003$, $p<0.001$ for hs-CRP), but this association disappeared for SAA. In addition, serum hs-CRP was positively correlated with leptin ($\beta=0.001$, $p=0.003$). Regression analysis showed that there was no association between serum Hcy, SAA, TBARS and SOD with serum leptin concentrations

Conclusion: Overall, the current study demonstrated that serum hs-CRP and SAA levels were independently correlated with BMI. Furthermore, serum hs-CRP was positively correlated with leptin. Focusing on such strategies may lead to promises for alleviating obesity and its co-morbidities.

Keywords: Leptin, Obesity, Oxidative stress, Inflammation

Introduction

Obesity is a chronic disease with different origins that derives from the interaction of social, behavioral, psychological, metabolic, cellular, and molecular factors (1). Reactive oxygen species (ROS) are produced under physiological conditions and responsible for direct or indirect tissue damages in many diseases. Thus, oxidative stress is involved in pathological processes such as obesity, diabetes, cardiovascular disease (CVD) and atherogenicity (2). Previous studies have shown that due to the depletion of antioxidant sources like superoxide dismutase (SOD), susceptibility to oxidative damage is even greater in obese subjects (3). SOD is an enzyme that participates in antioxidant defense in nearly all living cells exposed to oxygen(4). In the general population, thiobarbituric acid reactive substances (TBARS) concentrations are an important parameter of systemic oxidative stress (5), which is associated with various factors including age, body mass index (BMI), glucose and lipid metabolism (6).

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↑What is "already known" in this topic:
Oxidative stress in obesity is an important pathogenic mechanism of endothelial injuries caused by inflammation.

→What this article adds:
hs-CRP has a positive correlation with leptin concentration in obese people and is resulted in the effects of leptin induced inflammatory factors.

Surveys demonstrated subjects with visceral fat accumulation had significantly higher levels of circulating TBARS (5). Visceral adiposity correlates with systemic levels of oxidation; so it seems that fat accumulation parallels with ROS production (5). In addition, serum amyloid A (SAA) has produced from adipose tissue, offering a novel link between enhanced adipose tissue mass and the low-grade inflammatory state in which can be seen in obesity (7,8). On the other hand, although alteration in homocysteine (Hcy) levels has not been well-demonstrated in obese subjects, they lean to be suffering from metabolic syndrome (MS) signs. In turn it could be linked with increased levels of Hcy, as well as nutritional deficiencies of folate, pyridoxine or cobalamin that may lead to total higher levels of homocysteine in blood of obese subjects (9, 10). Also, high-sensitivity C-reactive protein (hs-CRP) which is raised in an inflammatory state, such as obesity, is seen as a strong independent predictor and risk factor for cardiovascular diseases (11).

In fact, there have been no published cross-sectional studies in Iran that have evaluated the association between leptin levels and the biomarkers of oxidative stress and inflammation among Iranian overweight and obese adults.

Therefore, in this study, we assessed the levels of TBARS, SOD (biomarkers of oxidative stress) and SAA, hs-CRP, homocysteine (inflammation biomarkers). Also, the relationship between circulating serum levels of leptin and BMI with biomarkers of oxidative stress and inflammation in overweight and obese people were evaluated.

Methods
Participants
This study was a cross-sectional study desgned and implemented in Tehran, Iran. Subjects were selected from people who referred to the clinical nutrition department in Iran University of Medical Sciences (IUMS). The individuals were excluded from the study if they had inflammatory or infectious diseases, cancer, secondary causes of obesity, pregnancy or breastfeeding, menopause, severe hepatic or renal disease and using medication, including anti-hypertensive drugs, hypoglycemic and hypolipidemic agents, using of NSAIDS or other inflammatory drugs and use of nutrition supplements. The study group comprised one hundred eighty-nine Iranian adult men and women aged 18-60 years old who were recruited for the study among the eligible ones. The participants were divided into three groups based on their BMI (kg/ m^2) : Normal group: 18.5<BMI<24.9, overweight: 25<BMI<29.5 and obese group: BMI >30. Sample size was calculated using the below formula (12):

$$N = \left[\frac{Z\alpha + Z\beta}{C}\right]^2 + 3$$
$$Z\alpha = 1.960$$
$$Z\beta = 0.842$$
$$C = 0.5 * ln[(1+r)/(1-r)] = 0.203$$
$$r\ (correlation\ coefficient) = 0.2$$

Ethics statements
This study was performed according to the principles of the Declaration of Helsinki and the study protocol (Ethic No: 90-03-27-14124) and was approved by the ethics committee of IUMS. Informed consent form and 24-hour recall were obtained from all subjects after explaining the purpose and nature of the study to them.

Assessment of anthropometric measurements
Body weight was measured to the nearest 0.1 kg on calibrated scales (OMRON, HBF-516-Japan). Height was quantified to the nearest 0.5 cm. The circumference of the hip was measured at the greatest protuberance of the buttocks and waist circumference was measured as a minimum circumference between the iliac crest and the rib cage while the participant's worn indoor clothes and barefoot. BMI was defined as weight in kilograms (kg) divided by height in meters squared (m^2).

Biochemical measurements
Fasting venous blood (10 mL) was collected from subject after fourteen hours of fasting period and kept in vacutainer tubes. Blood were divided into two heparinized and non heparinized tube. To separate serum, blood samples were immediately centrifuged. The samples were then stored at -80°C before analysis. Leptin concentrations were determined by the use of the ELISA Kit (eBioscience, USA) with inter- and intra-assay coefficient variances (CVs) of 6.7 and 8.9%, respectively and SOD was measured by the ELISA method using related kits (MyBioSource, USA) with inter- and intra-assay CVs of 6.7 and 8.9%, respectively. The levels of hs-CRP were assessed by the turbidometric method (Pars azmoon, Tehran, Iran) with inter- and intra-assay CVs of 6.7 and 8.9%, respectively. Plasma Hcy was assessed using high-performance liquid chromatography (HPLC) with colorimetric electrochemical detection with inter- and intra-assay CVs of 6.7 and 8.9%, respectively. TBARS levels were measured using the colorimetric technique as described by Uchiyama and Mihara (13) with inter- and intra-assay CVs of 6.7 and 8.9%, respectively and SAA levels were measured using high-sensitivity method (ELISA) with a BNII analyser (Dade Behring) with previously validated techniques (14) with inter- and intra-assay CVs of 6.7 and 8.9%, respectively.

Statistical analysis
Statistical analysis was performed using SPSS software (version 18.0; SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used for evaluation of normal distribution of quantitative variables. Descriptive statistics, Mean ± SD and median (Q1, Q3) were calculated for variables with normal and non-normal distribution, respectively. Logarithmic transformation was performed for some variables with non-normal distribution. We performed Pearson correlations and multiple regression analysis to assess the relationship between variables. TBARS, SAA, Hcy, hs-CRP and SOD were considered as dependent variables. Leptin and BMI were considered as independent variables. P value <0.05 was considered statistically significant.

Results

The study included 84 (44.4%) men and 105 (55.6%) women with an average age of 37.49±10.28 years. Mean weight and BMI were 79.62±17.96 kg and 29.16±5.8 kg/m², respectively. Data on the anthropometric characteristics of participants were presented in Table 1.

Our study demonstrated that serum hs-CRP (r=0.551, P<0.0001) and SAA levels (r=0.403, p<0.001) was significantly associated with BMI (Table 2). Pearson correlation analysis revealed that there was no association between Hcy, TBARS and SOD with BMI. Likewise, no association between Hcy, hs-CRP, SAA, TBARS and SOD with leptin was observed.

Simple linear regression analysis demonstrated that among the biomarkers of oxidative stress and inflammation in participants, serum hs-CRP and SAA were positively correlated with BMI (β=0.003, P<0.001 and β=7.83, p<0.001, respectively) (Table 3). This relationship remained significant even after controlling for other oxidative stress and inflammatory indicators for hs-CRP (β=0.003, p<0.001), but this association disappeared for SAA. In addition, serum hs-CRP was positively correlated with leptin (β=0.001, p=0.003). Regression analysis showed that there was no association between serum Hcy, SAA, TBARS and SOD with serum leptin concentrations.

Discussion

This study was aimed to assess the associations between biomarkers of oxidative stress and inflammation in overweight and obese subjects. The current study demonstrated that serum hs-CRP and SAA levels were associated with BMI among overweight and obese subjects. Our findings

Table 1. Baseline characteristics of participants

Variable	Mean ± SD	Median (Q1 , Q3)
Weight (kg)	79.62±17.96	
Height (cm)		163 (158, 173.25)
BMI (kg/m2)	29.16±5.8	
Waist circumference (cm)		99 (89, 109)
Wrist (cm)		17 (15.5, 18)
SBP (mmHg)		120 (110, 120)
DBP (mmHg)		80 (75, 80)
Hips (cm)	108.18±11.62	
Homocysteine (µmol/L)	18.09±7.28	
hs-CRP (mg/L)		0.02 (0.01, 0.06)
SAA (µg/mL)		157.9 (80.07, 225.77)
TBARS (mg/dL)		0.25 (0.18, 0.27)
Leptin (ng/mL)		9.2 (4, 19.2)

Mean ± SD was used for variables with normal distribution. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; WC, waist circumference; hs-CRP, high sensitivity- C reactive protein; TBARS, thiobarbituric acid reactive substances; SAA, serum amyloid A.

Table 2. Pearson correlation coefficients between biomarkers of oxidative stress and inflammation with BMI and leptin

Variables	BMI		Leptin	
	r	P[1]	r	P[1]
Homocysteine (µmol/L)	0.065	0.40	-0.042	0.59
hs-CRP (mg/L)	0.551	<0.001	0.12	0.12
SAA (µg/mL)	0.403	<0.001	0.026	0.77
TBARS (mg/dL)	0.019	0.86	0.06	0.57
SOD (U/mL)	0.0133	0.12	-0.035	0.68

hs-CRP, high sensitivity- C reactive protein; TBARS, thiobarbituric acid reactive substances; SAA, serum amyloid A; SOD, superoxide dismutase.
[1] Obtained from Pearson's correlation analysis.

Table 3. Multiple regression analysis between biomarkers of oxidative stress and inflammation with BMI and leptin

	BMI			Leptin		
	β	95% CI	P[1]	β	95% CI	P[1]
Homocysteine (µmol/L)						
Crude	0.079	-0.108, 0.267	0.40	0.009	-0.078, 0.096	0.84
Adjusted[2]	0.222	-0.133, 0.576	0.21	0.035	-0.075, 0.145	0.52
hs-CRP (mg/L)						
Crude	0.003	0.003, 0.004	<0.001	0.001	0.00, 0.001	0.003
Adjusted	0.003	0.002, 0.005	<0.001	0.000	-0.001, 0.00	0.65
SAA (µg/mL)						
Crude	7.83	3.99, 11.67	<0.001	0.219	-1.70, 2.14	0.82
Adjusted	2.08	-7.2, 11.36	0.65	-0.581	-3.40, 2.24	0.68
TBARS (mg/dL)						
Crude	0.00	-0.002, 0.003	0.87	0.00	-0.001, 0.001	0.91
Adjusted	-0.001	-0.005, 0.002	0.44	0.00	-0.001, 0.001	0.68
SOD (U/mL)						
Crude	0.001	0.00, 0.002	0.19	0.00	0.00-0.001	0.74
Adjusted	0.00	-0.002, 0.001	0.71	0.00	0.00-0.001	0.36

hs-CRP, high sensitivity- C reactive protein; TBARS, thiobarbituric acid reactive substances; SAA, serum amyloid A; SOD, superoxide dismutase.
[1] Obtained from multiple regression analysis.
[2] After controlled mutual effect of other biochemical indicators as covariates.

also indicated that serum hs-CRP levels were positively correlated with leptin.

Obesity has a dominant role in serum leptin levels (15). It has been proven that increased levels of serum leptin are accompanied with high BMI and the acute degree of obesity (15). But our results did not show any significant association between leptin and BMI ranges. Also in the present study, we failed to find an association between serum Hcy levels and BMI as well as leptin. Tungtrongchitr et al. (16) observed that there were higher levels of Hcy in overweight subjects compared with normal weight subjects. In addition, Vay´a et al. (10) demonstrated that morbidly obese patients had higher Hcy levels than non-obese subjects. In other words, this increase is related with anthropometric, inflammatory and metabolic parameters in obese people (10). In another study by Narin et al. (17) Hcy levels were increased in obese subjects with hyperleptinemia. Gallistl et al. (9) indicated that BMI was also significantly correlated with Hcy levels. Hcy has been implicated in promoting atherosclerotic and thrombotic vascular diseases (24). It has been revealed that Hcy can induce endothelial cell injury, which largely results in the generation of reactive oxygen species (16). Leptin resistance, which is associated with hyperleptinemia is common in obese individuals (18). Leptin induces oxidative stress in endothelial cells (19) and Hcy also seems to induce oxidative stress. So, an increase in Hcy levels was observed in obese subjects with hyperleptinemia (20). This inconsistency between our study and others may explain due to the differences in age groups, the health status of volunteers and evaluation methods.

Our data indicated that hs-CRP levels were positively correlated with BMI and leptin. Our findings were in consistent with previous results showing that BMI was the adiposity factor and strongly correlated with serum hs-CRP concentrations (21-23). In addition, visceral fat area and waist circumference were strongly correlated with hs-CRP levels (24). Our findings were in accordance with observed results by Sanip et al.(25). They have reported that hs-CRP levels were positively correlated with weight, BMI, waist circumference, hip circumference, body fat and visceral fat in obese females(25). The precise mechanism whereby obesity or adipose tissue accumulation contributed to elevated hs-CRP levels remain to be sustained; however, a solid relationship has been demonstrated between adipocytokines such as interleukin 6 (IL-6) and other inflammatory markers in obese women. It is also suggested that cytokines secreted by adipose tissue in obese subjects could play a role in the high secretion of inflammatory proteins in liver (26).

The results of our study indicated that serum SAA concentrations were associated with BMI. Several studies have reported that obese individuals tend to have increased levels of SAA compared with lean individuals, and they have also accounted that SAA expression is correlated with BMI and fat cell size (27-29). In a study by Lappalainen et al. (8) was observed that there was a relationship between serum SAA concentrations and leptin in obese subjects. Both SAA and leptin are predominantly expressed in adipocytes, which may in part explain this association (30). However,

our findings did not show any association between SAA and serum leptin concentrations. The findings of the current study did not support the research conducted by Poitou and Viguerie (31), who reported the overproduction of SAA in adipose tissue among obese subjects with a functional leptin receptor and leptin receptor-mutated subjects. The overexpression of SAA in leptin receptor-mutated subjects can be explained in part by the extreme early onset of adiposity as outgrows due to the absence of leptin signaling (31).

Surprisingly, this study did not show any significant relation between serum TBARS levels and BMI as well as leptin. Previous findings indicated that obese people have higher TBARS levels (32, 33). This inconsistency between our study and others may have been due to the differences in evaluation methods. TBARS assay is widely used as an indirect indicator of lipid peroxidation and can be applied for the measurement of oxidative stress (34). Low levels of antioxidants in obesity is associated with an increase in endogenous production of lipid peroxides as well as the depletion of antioxidant compounds in plasma (33). Okauchi et al. (5) demonstrated that subjects with visceral fat accumulation have high levels of TBARS.

Our results demonstrated that there was not any significant relation between serum SOD levels and BMI as well as leptin. In a study by Tungtrongchitr et al. (35) was seen that SOD activity was lower in overweight people compared with normal-weight individuals. Obesity is directly associated with high levels of leptin (36). Adverse effects of leptin may lead to the increase of oxidative stress, which is mediated by activation of NADPH oxidase (37).

Antioxidant enzymes play a major role in ROS scavenging (38). SOD is an important defensive antioxidant in nearly all living cells that is exposed to oxygen (39).

The potential feature of our study was the large sample size, but the limitation of this study was that this analysis was performed only in overweight and obese adults indicating that these results cannot be applied to healthy-weight adults. Another limitation of our study is the lack of measurement of visceral fat.

Conclusion

Overall, the current study demonstrated that serum hs-CRP and SAA levels were independently correlated with BMI. Furthermore, serum hs-CRP was positively correlated with leptin. Consequently, as enduring extent of obesity and its co-morbidities is increasing at an alarming rate, focusing on such strategies may lead to promises for alleviating non communicable diseases, by modifying the oxidative stress and inflammation biomarkers. Yet, further studies are needed to confirm our findings and determine possible underlying mechanisms.

Acknowledgements

Ethical approval
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent
Informed consent was obtained from all individual participants included in the study.

Conflict of Interests
The authors declare that they have no competing interests.

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