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ORIGINAL ARTICLE

Obese Subjects have Significantly Higher Serum Prooxidant-Antioxidant Balance Values Compared to Normal-Weight Subjects

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SUMMARY

Background: **It has been reported that obesity is associated with higher levels of oxidative stress. We aimed to evaluate the hypothesis that pro-oxidant antioxidant balance (PAB) values could be affected by adiposity and to assess the association between PAB levels and indices of obesity.**
Methods: **Subjects (n = 733) were recruited and then were divided into 3 groups of normal-weight (BMI ≤ 25, n = 207), overweight (25 < BMI ≤ 30, n = 375), and obese (BMI > 30, n = 151). PAB values were measured in all participants.**
Results: **There was a significant association between PAB values and weight when the correlation was determined for all subjects ($p < 0.05$). Obese subject had significantly higher levels of PAB values [40.8 (34.3 - 51.1) HK unit] compared with overweight [37.5 (29.7 - 47.3) HK unit] and normal-weight subjects [37.2 (29.6 - 45.2) HK unit] ($p < 0.05$ in each case). However, there was no significant difference in PAB values between normal and over-weight subjects ($p > 0.05$). On performing the analysis of covariance, low-density lipoprotein-cholesterol (LDL-C) was found to have a significant independent association with PAB values ($\beta = 0.046$, $p = 0.04$).**
Conclusions: **The high levels of PAB values in obese subjects without overt signs/symptoms of cardiovascular disease may be related to a heightened state of oxidative stress associated with obesity.**
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KEY WORDS

pro-oxidant-antioxidant balance, body mass index, obesity, adiposity, oxidative stress

LIST OF ABBREVATIONS

BMI - Body mass index
CVD - Cardiovascular disease
FBG - Fasting blood glucose
HDL-C - High-density lipoprotein-cholesterol
LDL-C - Low-density lipoprotein-cholesterol
PAB - Pro-oxidant-antioxidant balance
ROS - Reactive oxygen species

INTRODUCTION

Atherosclerosis is a chronic multi-factorial process that underlies the pathophysiology of cardiovascular disease (CVD). One of the modifiable risk factors for CVD is obesity, in which visceral obesity is an independent and modifiable risk factor.
Oxidative stress describes a situation where the production of reactive oxygen species (ROS) is greater than the ability of the tissue anti-oxidant defenses to neutralize the effects of ROS. This imbalance is usually related to the increased formation of ROS, leading to damage of the structural and functional integrity of cardiovascu-

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lar tissue and is thought to play a pivotal role in the pathogenesis of CVD and its complications. It has been suggested that oxidative stress may be a strong and independent prognostic predictor of cardiovascular events [1] and has been proposed as a significant risk factor for CVD. In patients with CVD, elevated levels of both oxidative stress status parameters [superoxide anion ($O2^{\bullet-}$) and malonaldehyde] and reduced protective activities of superoxide dismutase have been reported [2]. Moreover, oxidative stress may represent a common final pathway for several established CVD risk factors [2]. We have previously shown that one measure of oxidative stress, pro-oxidant antioxidant balance (PAB), is increased in patients with CVD and this was independent of the severity of the disease [3].

We [4,5] have previously shown that the elevation in serum anti-heat shock protein IgG levels is associated with indices of body mass in healthy subjects. Thus, in the present study, we aimed to evaluate the hypothesis that PAB values could be affected by obesity and to assess the association between its levels and indices of obesity, as a risk factor for CVD, and PAB levels in patients without overt clinical CVD.

MATERIALS AND METHODS

Subjects

Seven-hundred and thirty three subjects, including normal-weight (n = 207), overweight (n = 375), and obese subjects (n = 151), were recruited from the staff of the Shahid-Hasheminejad Gas-Processing Company (S.G. P.C), Sarakhs, Iran. They were all male subjects aged between 20 - 69 years with body mass index (BMI) between 25 - 45 kg/m^2. In this study overweight was defined as a BMI of 25 to < 30, and a BMI of ≥ 30 was defined as obese. None of the subjects had undertaken any weight control measures nor had they had any significant medical history or taken drug therapy within the 3 months previous to their participation in the study. Participants were provided with information about the study by both verbal explanation and written information sheets. Those who had exclusion criteria such as poorly control diabetes, severe hypertension, overt signs/symptoms of CVD, endocrine abnormalities, or who preferred not to participate at any point were withdrawn from the study. Each patient gave informed written consent to participate in the study, which was approved by the Mashhad University of Medical Science Ethics Committee.

Blood sampling and routine biochemical analysis

Blood samples were taken from each patient for analysis after 12 hours fasting. Following venepuncture, blood samples were collected into Vacutainer® tubes and centrifuged at 5,000 g for 15 minutes at 4°C. After separation, aliquots of serum were frozen at -80°C until analysis. A full fasted lipid profile comprising total cholesterol, triglycerides, fasting blood glucose (FBG), high density lipoprotein cholesterol (HDL-C,) and low-density lipoprotein cholesterol (LDL-C) was determined for each subject. Serum lipid and fasting blood glucose concentrations were measured enzymatically with the use of commercial kits using the BT-3000 autoanalyzer (Biotechnica, Rome, Italy).

Anthropometric and other measurements

Anthropometric parameters were measured twice, using a standard mercury sphygmomanometer. The BMI was calculated as weight (kg) divided by height squared (m^2).

Chemicals used for pro-oxidant-antioxidant balance (PAB) assay

TMB powder (3,3′,5,5′-tetramethylbenzidine, Fluka), peroxidase enzyme (Applichem: 230 U/mg, A3791, 0005, Darmstadt, Germany), chloramine T trihydrate (Applichem: A4331, Darmstadt, Germany), hydrogen peroxide (30%) (Merck). All the other reagents used were reagent grade and were prepared in double distilled water [6].

Pro-oxidant-antioxidant balance (PAB) assay

In order to measuring the PAB values, serum samples were used. A modified PAB was applied based on a previously described method [3,7]. The standard solutions were prepared by mixing varying proportions (0 - 100%) of 250 μM hydrogen peroxide with 3 mM uric acid (in 10 mM NaOH).

Sixty-milligram TMB powder was dissolved in 10 mL DMSO; for preparation of TMB cation, 400 μL of TMB/DMSO was added to 20 mL of acetate buffer [0.05 M buffer, pH 4.5], and then 70 μL of fresh chloramine T (100 mM) solution was added into this 20 mL, mixed well, incubated for 2 hours at room temperature in a dark place; 25 U of peroxidase enzyme solution was added to 20 mL TMB cation, dispensed in 1 mL aliquots and stored at -20°C; in order to prepare the TMB solution, 200 μL of TMB/DMSO was added into 10 mL of acetate buffer [0.05 M buffer, pH 5.8]; the working solution was prepared by mixing 1 mL TMB cation with 10 mL of TMB solution, incubated for 2 minutes at room temperature in a dark place and immediately used. Ten μL of each sample, standard or blank (distilled water) was mixed with 200 μL of working solution, in each well of a 96 well plate, which was then incubated in a dark place at 37°C for 12 minutes; at the end of the incubation time, 100 μL of 2M HCl was added to each well and measured in an ELISA reader at 450 nm with a reference wavelength of 620 or 570 nm. A standard curve was provided from the values relative to the standard samples. The values of the PAB are expressed in arbitrary HK units, which represent the percentage of hydrogen peroxide in the standard solution. The values of the unknown samples were then calculated based on the values obtained from the above standard curve.

Table1. Comparison of anthropometric and biochemical parameters of obese, overweight, and normal weight subjects.

	Normal weight	Overweight	Obese	p-value
Age (year)	43 (34 - 49)	45 (35 - 50)	47 (42 - 51)	< 0.001
BMI (kg/cm^2)	22.86 ± 1.96	27.39 ± 1.38	32.35 ± 2.53	< 0.001
Height (cm)	171 (167 - 175)	171 (167 - 175)	170 (165 - 173)	0.014
Weight (kg)	68.33 ± 8.46	80.21 ± 7.82	92.37 ± 1.00	< 0.001
FBG (mg/dL)	91 (86 - 97.75)	94 (88 - 102)	97 (91 - 107)	< 0.001
Cholesterol (mg/dL)	177.94 ± 33.98	183.81 ± 35.78	189.14 ± 38.31	0.013
LDL-C (mg/dL)	124.21 ± 34.77	127.10 ± 33.15	135.14 ± 34.22	0.01
HDL-C (mg/dL)	41.2 (34.2 - 47.5)	38.4 (33.9 - 43.9)	37.9 (33.1 - 42.1)	0.009
TG (mg/dL)	105.50 (76 - 161.75)	135.50 (98 - 192.75)	150 (112 - 203)	< 0.001

BMI, body mass index; WC, waist circumferences; HC, hip circumferences; FBG, fasting blood Glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. Values are expressed as mean ± SD, or median and interquartile range. α and * indicates comparison with control and overweight group respectively. (α, *: p < 0.05). Chi-square, one-way analysis of variance (ANOVA), Kruskal-Wallis tests were used to compare qualitative and quantitative (normal and non-normal) variables, respectively.

Table 2. Correlations between serum PAB values with other anthropometric and biochemical parameters.

Parameter	Normal weight		Overweight		Obese		Groups	Combined
	r	p-value	r	p-value	r	p-value	r	p-value
Age	0.016	0.822	-0.015	0.767	-0.044	0.592	0.012	0.746
Height	0.029	0.674	0.084	0.103	-0.007	0.934	0.035	0.344
Weight	-0.051	0.466	0.099	0.057	0.057	0.484	0.093	0.011*
BMI	-0.030	0.668	0.050	0.335	0.055	0.499	0.090	0.014*
LDL-C	0.212	0.002*	-0.018	0.724	0.013	0.876	0.068	0.065
Cholesterol	0.122	0.081	-0.046	0.375	0.003	0.974	0.24	0.509
FBG	-0.064	0.360	-0.042	0.414	-0.007	0.928	-0.017	0.636
Triglyceride	0.023	0.742	-0.070	0.174	0.004	0.961	-0.009	0.808
HDL-C	0.081	0.249	0.017	0.746	-0.059	0.477	0.013	0.732

BMI; body mass index, FBG; fasting blood glucose, HDL-C; high-density lipoprotein-cholesterol, LDL-C; low-density lipoprotein-cholesterol. Correlations were assessed using Spearman's correlation coefficients. There were no significant correlations.

Statistical analysis

Data were expressed as means ± SD (for parameters with a normal distribution) or median and interquartile range (in the case of non-normally distributed data). For parameters with a normal distribution group comparisons were performed using ANOVA (for ≥ 3 groups). For parameters with a non-normal distribution Kruskal–Wallis (for ≥ 3 groups) test was used. Categorical data were compared using Chi-square test. A two-sided p value < 0.05 was considered statistically significant. Bivariate correlations between different parameters and PAB values were performed using Spearman's rank correlation. Analysis of covariance was performed to investigate the possible impact of different parameters on PAB-values.

RESULTS

Demographic data

Among the 733 subjects who were involved in the study, 207 subjects were of normal-weight, 375 subjects were over-weight and finally 151 subjects were entered as the obese group. Age was significantly different among the subgroups (p < 0.001). As would be expected weight, FBG, and lipid profile was significantly dif-

ferent between the 3 subgroups ($p < 0.05$). (Triglyceride, HDL-C, and LDL-C were significantly different between control subjects and overweight and obese subjects). Table 1 shows the summarized data of biochemical parameters in different subgroups.

PAB values among the 3 groups

Comparisons between the 3 subgroups revealed that the PAB values were significantly higher in obese cases [40.8 (34.3 - 51.1) HK unit] compared with normal-weight [37.2 (29.6 - 45.2) HK unit] ($p = 0.015$) and overweight subjects [37.5 (29.7 - 47.3) HK unit] ($p = 0.008$). However, there was no statistical difference in PAB values between normal and over-weight subjects ($p = 0.904$). After adjustment for age, the significant difference in PAB values remained among the 3 groups.

Correlations

Spearman's correlation test was used to evaluate the possible association between PAB values in different subgroups with biochemical parameters. The analysis showed that PAB values were not related to any biochemical factor such as FBG, total-cholesterol, HDL-C, and triglyceride ($p > 0.05$) in any of the three groups. Significant correlation was found between serum PAB and LDL-C in the normal weight group ($r = 0.212$, $p = 0.002$). When all groups were combined there was a significant association between PAB values and weight ($r = 0.093$, $p = 0.011$) and BMI ($r = 0.090$, $p = 0.014$) (Table 2).

Analysis of covariance

Analysis of covariance was performed to investigate the possible impact of different parameters on PAB-values. The results showed that LDL-C ($\beta = 0.046$, $p = 0.04$) was independently associated with PAB values.

DISCUSSION

The principal finding of the present study was the significant elevation in PAB values in obese subjects, indicating a positive correlation between obesity and increased oxidative stress levels. Our results are concordant with previous studies that have reported higher levels of oxidative stress in obese subjects in comparison with healthy ones [8,9].

The lack of significant correlation between individual coronary risk factors and PAB values in our survey is consistent with previous studies in which oxidative stress was found to be an independent risk factor for cardiovascular events and cardiac death [1,10]. Similar results have been reported previously using the PAB method in patients with acute coronary syndrome [6]. However, when all groups were combined, we found a significant association between PAB values and weight and BMI, indicating a significant association with PAB values, a marker of oxidative stress and adiposity.

The determination of PAB has yet to be used in routine clinical practice, primarily because of the lack of a universally accepted method. Different methods have been developed that can measure the total pro-oxidant and antioxidant capacities separately. We have presented a simple, rapid, and cost-effective method (the PAB assay), which can measure the pro-oxidant burden and the antioxidant capacity in one assay, giving a redox index [11].

In conclusion, the high levels of PAB values in obese subjects without overt signs/symptoms of CVD or established CVD may be related to a heightened state of oxidative stress associated with obesity. This may be explained by the elevation in oxidative stress which develops relatively early in the atherogenic process; however, this should be confirmed in a prospective study. Early medical intervention such as statin therapy in patients with high oxidative levels may be useful in reducing the risk of CVD [12].

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Declaration of Interest:

None of the authors have a conflict of interest.

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