Introduction

Over the last two decades, overweight and obesity have become major threats to public health worldwide. Overweight and obesity have become major threats to public health worldwide. Of particular concerns are emerging data showing that the obesity epidemic is not confined to developed countries but is starting to affect many developing countries. Furthermore, obesity is a major risk factor for important illnesses, including hypertension, type-2 diabetes, dyslipidemia, and cardiovascular disease (CVD).

It has been reported that oxidative stress (OS) may play a critical role in the pathogenesis and development of obesity-related diseases, in particular CVDs, hypertension, and type-2 diabetes. Although the exact biochemical mechanisms responsible for the association between obesity and the above-mentioned diseases are not fully understood, it is known that the increased production of reactive oxygen species (ROS) at high levels is associated with cellular damage, including the oxidation of cell membranes and proteins. Furthermore, accumulating evidence from animal studies has shown that OS is involved in the development of obesity-related diseases.

Total Antioxidant Capacity, Lipid Peroxidation, and Erythrocyte Antioxidant Enzyme Activities in Subjects with Obesity: A Case-Control Study

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Abstract

Introduction: Considering that the prevalence of obesity has increased dramatically in recent years, one of the key targets of public health is obesity and its associated pathological conditions. Recent evidence suggests that oxidative stress (OS) may be the mechanistic link between obesity and the development of metabolic and vascular diseases related to obesity. In this study, it was hypothesized that obesity would be associated with lipid peroxidation (LP) and antioxidant enzyme activities in erythrocytes.

Methods: In this case-control study, 80 subjects with obesity and 80 age- and gender-matched subjects with normal weight were selected from the health centers affiliated with Zabol University of Medical Sciences, Iran. General information was gathered from each participant using questionnaires. Serum malondialdehyde (MDA) and total antioxidant capacity (TAC), as well as, superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT) activities underwent assessment.

Results: The study sample consisted of 160 obese adults (42.5% males and 57.5% females, with a mean age of 41.0±7.6 years). Serum MDA levels (P=0.016) and erythrocyte SOD activity (P=0.013) were significantly higher in subjects with obesity compared to non-obese controls. Moreover, subjects with obesity had significantly lower serum TAC (P=0.008) in comparison to the controls. There were no significant differences in erythrocyte GPX and CAT activities between subjects with obesity and non-obese controls. Significant positive correlations were observed between body mass index (BMI) and serum MDA levels (r=-0.461, P<0.001) and erythrocyte SOD activity (r=-0.442, P=0.002). Furthermore, a significant inverse correlation was found between BMI and serum TAC levels (r=-0.426, P=0.008).

Conclusion: The present findings provide further evidence suggesting that obesity leads to substantial LP and OS, which, in turn, may contribute to the development of obesity-related diseases.

Keywords: Obesity, Oxidative stress, Lipid peroxidation, Antioxidant enzymes
and human studies indicates that obesity is associated with increased myocardial OS and lipid peroxidation (LP). OS can be defined as the imbalance between free radical damage (e.g., LP) and antioxidant protection. Nevertheless, mechanisms contributing to increased free radical production in obesity are not well understood. Considering that previous studies suggest that OS is a state of imbalance between the production of ROS and antioxidant defenses, we hypothesized that reduced antioxidant defenses, either due to reduced enzymatic or non-enzymatic antioxidants, may contribute to increased ROS and related diseases in obese subjects.

The determination of total antioxidant capacity (TAC) is now considered a tool in the medical diagnosis and treatment of several diseases, including CVD and diabetes mellitus. In serum, antioxidant molecules involved in free radical scavenging include endogenous (e.g., uric acid, albumin, and circulating thiols) and exogenous (e.g., vitamins E and C) antioxidant molecules. Previous studies have suggested that plasma TAC levels change under OS conditions. Therefore, plasma TAC levels may be altered by obesity.

Enzymatic antioxidants, such as copper-zinc superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT), protect cells against the harmful effects of free radicals by scavenging or inhibiting their formation. On the other hand, decreased activity of these enzymes may also contribute to increased OS.

The available data regarding the association between obesity and LP, as well as erythrocyte antioxidant enzyme activities, are limited, and the existing data are highly controversial. Therefore, the aim of this study was to investigate the association between obesity and serum malondialdehyde (MDA) concentrations as a powerful marker of LP and the main antioxidant enzymes, including SOD, GPX, and CAT, in the erythrocytes of apparently obese subjects.

Materials and Methods

Study Participants

In this case-control study, 80 subjects with obesity and 80 age- and gender-matched healthy normal weight controls were voluntarily recruited from the health centers affiliated with Zabol University of Medical Sciences, Iran, from November 2019 to March 2020. Cases and controls were selected based on specific body mass index (BMI) levels.

The inclusion criteria were a willingness to participate in the study, age range between 20 and 60 years old, and BMI ≥ 30 kg/m² and BMI < 25 for subjects with obesity and healthy normal weight (non-obese) controls, respectively. On the other hand, the exclusion criteria included being pregnant, breastfeeding, consuming any antioxidant supplements within the previous three months, being on weight loss diets for at least three months prior to participation in the study, receiving cholesterol-lowering medication, estrogen, progesterone, or diuretics. The other exclusion criteria were having a history of acute or chronic liver diseases, kidney dysfunctions, diabetes, and other endocrine disorders, rheumatoid arthritis, CVD, thyroid disorders, autoimmune, or endocrine disorders, and performing chemotherapy during the previous year.

The objectives and protocol of the study were fully clarified for the participants, and then they were asked to sign a written informed consent form. The whole study was planned according to the ethical standards detailed in the Declaration of Helsinki.

Anthropometric Measurements

Weight was measured with light clothes and without shoes to the nearest 0.1 kg using a digital scale (Seca 840; Seca GmbH, Hamburg, Germany). Height was measured without shoes with a precision of 0.1 cm by using a wall-mount measuring tape. BMI was calculated as weight (kg) divided by the squared height (m²). Waist circumference (WC) was determined as the midpoint between the lowest ribs and the superior border of the iliac crest while the subject was in a standing position and after expiration with an inelastic measuring tape to the nearest 0.1 cm. To decrease subjective error, all measurements were performed by the same trained technician.

Body Composition and Physical Activity Assessment

The percentage of body fat mass (%) and visceral fat level were assessed using a bioelectrical impedance analysis system (InBody S10, JMWW140, Korea). For increasing accuracy, participants were advised to refrain from moderate or intense exercises for 1–2 hours before using bioelectrical impedance analysis and to urinate before testing.

Physical activity levels were calculated based on the data obtained from the short form of the International Physical Activity Questionnaire and then categorized as “light”, “moderate”, and “high” activity.

Laboratory Measurements

To measure laboratory parameters, 10 mL of venous blood was drawn from the antecubital vein after overnight fasting (10–12 hours). The sera were separated by centrifugation at 3500 rpm (~2000 g) for 10 minutes and immediately transferred into newly labeled polypropylene tubes. To assay erythrocyte SOD, GPX, and CAT activities, the whole blood samples were also rinsed three times with cold saline (9.0 g/L NaCl) and hemolyzed by the addition of an equal volume of ice-cold demineralized ultrapure
water. Both sera and hemolysates were immediately stored at -80 °C until the analysis time.

Serum TAC concentrations were determined colorimetrically in triplicate samples using 2, 2'-Azino-di-[3-ethylbenzthiazoline sulphonate] (ABTS).\(^7\) The assay relies on the ability of antioxidants in the sample to inhibit the oxidation of ABTS to ABTS\(^{+}\) by a peroxidase. The amount of the produced ABTS\(^{+}\) can be monitored by reading the absorbance at 600 nm. The serum concentrations of MDA were also assessed by the measurement of thiobarbituric acid reactive substances according to the method of Uchiyama and Mihara.\(^8\)

The activity of erythrocyte SOD (EC 1.15.1.1) was measured by the Ransel kit (Randox Laboratories, Ltd., UK, Cat. No. SD-125). In addition, erythrocyte GPx (EC 1.11.1.19) activity was determined by the Ransel kit (Randox Laboratories, Ltd., UK, Cat. No. RS-504). Erythrocyte CAT (EC 1.11.1.6) activity was measured by the method of Abei\(^9\) following the decomposition of H\(_2\)O\(_2\) in phosphate buffer with a pH level of 7.2 spectrophotometrically at 230 nm. One unit of CAT is defined as the amount of enzyme that liberates half of the peroxide oxygen from an H\(_2\)O\(_2\) solution in 100 seconds at 25 °C.

**Statistical Analysis**

All statistical analyses were performed using SPSS software (version 18; SPSS, Chicago, IL, USA). The normality of the data distribution was checked using a Q–Q plot and the Kolmogorov–Smirnov test. The results were presented as means ± standard deviations for normally distributed quantitative data and frequencies (percentage) for qualitative data. For the comparison of the general characteristics among the subjects with obesity and healthy controls, an independent sample t test and a Pearson chi-square test were used for quantitative and qualitative variables, respectively. An independent sample t test was utilized to investigate the differences in the OS markers between subjects with obesity and normal-weight controls. Correlations between normally distributed quantitative variables were evaluated by the calculation of Pearson’s correlation coefficients, and P values less than 0.05 were considered statistically significant.

**Results**

In total, 160 participants were included in the study (68 males [42.5%] and 92 females [57.5%]). The baseline characteristics of the study subjects are presented in Table 1. Compared with the non-obese group, obese subjects had higher values of weight, BMI, WC, and fat mass (P < 0.001 for all). Because of the study design, controls and subjects with obesity were almost identical in terms of age, gender, and physical activity levels.

The comparison of OS markers in non-obese controls and subjects with obesity is provided in Table 2. The serum levels of MDA (P = 0.016) and erythrocyte SOD activity (P = 0.013) were significantly higher in subjects with obesity compared to non-obese controls. Based on the results, subjects with obesity had significantly lower serum TAC (P = 0.008) when compared to the controls. There were no significant differences in erythrocyte GPx and CAT activities between subjects with obesity and non-obese controls.

The correlations of BMI with OS markers and erythrocyte antioxidant enzyme activities in all the subjects were summarized in Table 3. Significant positive correlations were found between BMI and serum MDA levels (r = -0.461, P < 0.001) and erythrocyte SOD activity (r = -0.442, P = 0.002). Furthermore, a significant inverse correlation was observed between BMI and serum TAC levels (r = -0.426, P = 0.008).
Discussion

The present study evaluated the relationships between obesity and the serum levels of MDA and TAC, as markers of OS, as well as the activity of the main antioxidant enzymes, including SOD, GPX, and CAT. According to the findings, subjects with obesity exhibit a substantial OS condition, as evidenced by the higher serum levels of MDA when compared with values found in non-obese controls. This OS condition occurs concomitantly with a noticeable increase in erythrocyte SOD activity and a significant decrease in the serum levels of TAC.

Obesity has been recognized as a major underlying factor in the pathogenesis of a large number of health disorders, including diabetes, cardiovascular complications, and cancer. Several mechanisms are involved in enhancing OS in obesity. It has been found that obesity is associated with low-grade chronic systemic inflammation in adipose tissue. This condition promotes pro-inflammatory status, which is strongly related to OS. Excessive fat accumulation in subjects with obesity also leads to an increase in serum FFA levels, which, in turn, cause OS due to increased mitochondrial uncoupling and β-oxidation, leading to the increased production of ROS.

Despite extensive evidence indicating that obesity is a state of OS, the mechanism contributing to increased ROS production in obesity remains elusive. During the past years, previous studies have reported several mechanisms demonstrating that obesity can increase the production of ROS. For example, Vincent et al. reported increased mechanical and metabolic load on the myocardium, thus increasing myocardial oxygen consumption. A negative consequence of such increased myocardial oxygen consumption is the production of ROS such as superoxide and hydrogen peroxide from increased mitochondrial respiration. Our findings conform to those of other studies, confirming increased OS in central fat distribution. Furukawa et al. concluded that plasma adiponectin levels are negatively related to OS levels and that probably increased OS in accumulated fat leads to decreased production of adipocytokines.

In this study, a positive association was observed between obesity and OS levels; thus, it was attempted to identify factors associated with increased OS. Our results indicated that erythrocyte GPX and TAC activities were similar in obese subjects and non-obese control subjects, whereas erythrocyte SOD activity was significantly increased compared with controls. Indeed, our findings do not support the idea that obesity reduces the activities of primary antioxidant enzymes, including SOD. These results are in line with previous reports, demonstrating up-regulated SOD in animal models of obesity. The positive correlation between obesity and the erythrocyte SOD activities observed in subjects with obesity may indicate a compensatory response to increased ROS production. This correlation was not significant for erythrocyte GPX activities, likely due to the depletion of cellular glutathione as the main substrate for GPX in response to increased ROS. However, these controversial findings may be partially due to differences in grades and duration of obesity, the applied laboratory methods, and/or sample size.

Two possible factors may contribute to increased OS, including increasing free radical generation and declining the enzymatic and/or non-enzymatic antioxidant defense systems. Therefore, we studied the activities of erythrocyte antioxidant enzymes and serum TAC levels in the participants. The serum levels of each antioxidant can be measured separately in the laboratory, but measurements are time-consuming, labor-intensive, and costly. Considering that the effects of the antioxidant components in plasma are additive, the measurement of TAC can accurately reflect the redox status of the serum. The results of our study indicated that the antioxidant defense system is compromised in obesity, as evidenced by increased MDA levels and decreased levels of TAC in the serum. This finding corroborates previous reports regarding decreased plasma TAC levels in obesity. Furthermore, Suzuki et al. found decreased serum levels of carotenoids (as antioxidant components in the serum) in women with abdominal adiposity. Decreased serum TAC levels in obesity and fat accumulation may indirectly indicate whole free radical activity.

Some points should be considered in interpreting our findings. First, due to the case-control design of the study, one cannot infer causality. Therefore, our findings need to be confirmed in future prospective studies. Second, the associations and significant differences could be in part explained by other underlying risk factors not controlled for in this study, including unhealthy lifestyle behaviors and dietary habits. Third, we could not assess the metabolic syndrome status, lipid profile, C-reactive protein, plasma glucose levels, estrogen levels, and nutrient intakes (including antioxidant nutrients) of participants. However, the panel of biomarkers used in this study was fairly comprehensive regarding antioxidant status.
Conclusion

In general, the present study provides further evidence suggesting that obesity leads to substantial LP and OS, thus contributing to the development of obesity-related diseases. Based on the results of the current study, the use of antioxidant supplements or the consumption of nutrients with a high level of anti-oxidative components might be useful in preventing oxidative damage in subjects suffering from obesity.

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Competing Interests

The authors declare that they have no conflict of interests.

Ethical Approval

The study proposal and procedures were approved by the Ethics Committee of Zabol University of Medical Sciences (Approval No. IR.ZBMU.REC.1398.133).

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