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Research Article
Evaluation of Oxidant-antioxidant Status in Obese Children and Adolescents

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Abstract

Objective: This study aimed to evaluate the association between endogenous antioxidants and oxidative stress and selected risk factors in obese children without co-morbidities. **Methodology:** A total of 121 school children (58 obese and 63 normal weight), aged between 10 and 15 years old were recruited from public schools in Amman, Jordan. Levels of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and malondialdehyde (MDA) concentration was used as a biomarker for oxidative stress were measured. Fasting Blood Glucose (FBG) and lipids levels were determined in serum and anthropometric parameters were also measured. **Results:** The SOD activity was significantly higher in obese children relative to normal weight (190.5±39.5 and 144.1±44.8, respectively; $p<0.05$) and was correlated with BMI ($r = 0.456$). However, GPx and CAT activities were not affected by an increase in BMI ($p>0.05$). Meanwhile, low density lipoprotein cholesterol (LDL-c) levels were significantly correlated with SOD activity ($r = 0.330$). The MDA levels were significantly higher in obese children relative to normal weight children (4.62±1.15 vs 3.58±0.64, respectively; $p<0.001$) and was correlated with BMI ($r = 0.531$), triglycerides ($r = 0.315$), LDL-c ($r = 0.378$) and SOD activity ($r = 0.328$) ($p<0.05$). Both MDA levels and SOD activity were correlated with waist circumference ($r = 0.453$ and $r = 0.322$, respectively; $p<0.05$) and hip circumference ($r = 0.487$ and $r = 0.369$, respectively; $p<0.05$). **Conclusion:** This study concluded that an increase in oxidative stress in obese Jordanian school children is associated with increased MDA levels which reflects an imbalance between reactive oxygen species production and antioxidant defense responses as evidenced by the increased activity of the early antioxidant response enzyme SOD that was associated with increased BMI.

Key words: Antioxidant enzymes, obesity, oxidant stress, school children

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Childhood obesity causes significant morbidity and mortality. Obesity is a major global health problem that is reaching pandemic levels in both developed and developing countries[1]. Between 1980 and 2013, the prevalence of overweight and obese in children and adolescents (<20 years) increased from 8.1-12.9% in boys and from 8.4-13.4% in girls. In developed countries, 24% of boys and 23% of girls are obese or overweight. In Jordan, a low-middle income country, more than 24% of boys and 25.4% of girls (<20 years) were either overweight or obese and 8.0% were obese[2].

Risk factors for obesity-related cardiometabolic disorders are becoming increasingly prevalent in children and adolescents[3]. As such, the presence of oxidative stress could be a major factor in obesity-related cardiometabolic conditions[4,5]. Increased muscle activity needed to carry excess body weight, elevated lipid levels and hyperleptinemia as well as chronic inflammation and oxidation of fatty acid are all possible contributors to the generation of free radicals and oxidative stress in obese individuals[6,7].

Determination of childhood obesity physiopathology should be an initial step in strategies to prevent obesity and its complications in children and adolescents. However, studies that examined the possible mechanisms of cellular responses to oxidative stress damage caused by obesity and their influence in many cardiometabolic illnesses related to obesity often involved only adult populations. There are few studies on the response to obesity-related oxidative stress damage in children and adolescents[8,9]. Moreover, there are no data concerning oxidant-antioxidant status in obese children and adolescents in Jordan. As such, this study sought to evaluate the activities of several antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and the concentration of the lipid oxidation product malondialdehyde (MDA) in Jordanian school children who were either of normal body weight or obese. The study also investigated the correlation between oxidative stress and antioxidant enzymes with anthropometrical parameters of the study group.

MATERIALS AND METHODS

This study was undertaken between March, 2015 and January, 2016. A total of 58 obese (28 boys and 30 girls) and 63 normal weight (31 boys and 32 girls) schoolchildren (10-15 years old) were recruited from four public schools that were randomly selected from a list of 20 public schools provided by the Ministry of Education, Jordan. The selected schools were visited and consent forms were sent to parents. Parents who consented to have their children participate were asked to complete a health questionnaire. Students who had chronic or acute diseases, who were receiving any medical treatments (e.g., antioxidant vitamins such as ascorbate, tocopherols or α-carotene), or those with endogenic obesity or genetic syndromes were excluded from the study[8,9]. The study protocol was approved by the Research Review Committee, Deanship of Scientific Research, University of Jordan, Ministry of Education, Jordan.

In a follow-up visit, consent was obtained from children and adolescents, height, weight, waist circumference (WC) and hip circumference (HC) were measured using standard procedures. Body Mass Index (BMI) and waist to hip ratio (WHR) were also calculated. World Health Organization (WHO) BMI-for-age criteria were used to define obese and normal weight participants. Blood samples were taken during a school session after the students had fasted overnight for 12 h. Samples were collected into serum vacutainers and ethylene diamine tetraacetate (EDTA) tubes. Erythrocytes were washed according to the method described by Ivanov[10] with minor modifications. Both serum and erythrocyte aliquots were stored at 20°C until analysis.

Serum total cholesterol (TC), triglyceride (TG) and high-density lipoprotein cholesterol (HDL-c) levels were determined with a enzymatic colorimetric assay provided in commercial kits from BIOLABO (Maizy, France). Low-density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL-c) levels were calculated using the Friedewald[11] formula. Fasting Blood Glucose (FBG) was measured using a glucose oxidase method (BioSystems, Barcelona, Spain). Absorbance for these assays was taken at 500 nm.

Superoxide dismutase activity was measured according to methods described by Beauchamp and Fridovich[12] and Murthy *et al.*[13]. Units of SOD activity were expressed as the amount of enzyme required to inhibit Nitroblue Tetrazolium reduction by 50%. The SOD activity was estimated in U mg^{-1} Hb. Absorbance was read at 560 nm. Catalase activity was estimated according to the method of Aebi[14]. Catalase activity was expressed as U mg^{-1} Hb, 1 U of CAT activity represented the decomposition of 1 μmol hydrogen peroxide in 1 min at 37°C. Absorbance was read at 240 nm. Glutathione peroxidase (GPx) activity was measured using the method described by Paglia and Valentine[15]. One unit of GPx activity was defined as 1 μmol of nicotinamide adenine dinucleotide phosphate (NADPH) oxidized per minute. One unit of enzyme was defined as 1 μmol of NADPH oxidized per minute; absorbance was read at 340 nm. The amount of lipid

peroxidation expressed as MDA concentration was estimated by the double heating method described by Draper and Hadley[16]. Absorbance for these assays was read at 532 nm. A UV-Vis Double PC UVD-2950 spectrophotometer was used to measure absorbance in all of the assays (Labomed, Inc., Los Angeles, USA).

Statistical analysis: Statistical analysis was performed using SPSS version 17 (SPSS for Windows, Rel. 17.0.1. 2008 Chicago: SPSS Inc). Data are presented as Means±SD. Differences in mean values were evaluated by one-way analysis of variance (ANOVA). The Pearson's model was used to test associations between the considered variables. Statistical significance was defined as $p<0.05$.

RESULTS AND DISCUSSION

Table 1 shows the mean age of the obese and normal weight students was (12.52±1.14 and 12.11±1.24 years, respectively; $p>0.05$). In general, there were significant differences between the obese and normal weight groups with regard to all examined anthropometric indicators (Table 1, $p<0.001$). The mean serum TC, TG, LDL-c and VLDL-c levels were significantly higher in obese subjects than in their normal weight counterparts ($p<0.01$), whereas, there was no significant difference in HDL-c levels or FBG (85.49±13.07 and 86.27±11.75, respectively; $p<0.05$) between obese and normal weight students.

The SOD activity was significantly higher in obese children and adolescents than in normal weight students (Fig. 1b; 190.5±39.5 and 144.1±44.8, respectively; $p<0.05$). In contrast, the mean activities of the antioxidant enzymes GPx and CAT (Fig. 1b, GPx: 18.65±4.88 and 17.12±4.77, respectively; $p<0.05$ and CAT: 39.95±6.22 and 37.96±9.49, respectively; $p<0.05$) were not affected by the increased BMI ($p>0.05$). Moreover, MDA levels were significantly higher in obese children relative to normal weight subjects (Fig. 1a, 4.6261±1.152 and 3.58±0.64, respectively; $p<0.001$).

The SOD activity was significantly positively correlated with BMI, LDL-c, TG, HC and WC (Table 2, $p<0.01$) values. Although, BMI was strongly positively correlated with SOD activity ($r = 0.456$, $p<0.01$), no significant association was found between GPx and CAT activity and all tested variables (Table 2, $p>0.05$). The BMI, WC, HC, TG and LDL-C were significantly positively correlated with MDA levels (Table 3). The BMI levels were also strongly correlated with MDA levels (Table 3, $r = 0.531$, $p<0.01$), but there was no significant correlation between MDA levels ($p>0.05$) with TC ($r = 0.233$), HDL-c ($r = 0.036$) and glucose ($r = 0.074$).

Table 1: Serum lipid concentration, fasting blood glucose levels, obesity indices in a group of obese, normal weight children and adolescents in Jordan

Indicators*	Obese (n = 58)	Normal weight (n = 63)	p†
Boys/girls	28 (48.3)/30 (51.7)	31 (49.2)/32 (50.8)	
Age (years)	12.52±1.14	12.11±1.24	0.065
Weight (kg)	68.59±16.55	42.08±9.53	0.001
BMI (kg m^{-2})	28.11±4.09	18.30±2.18	0.001
WC (cm)	86.06±12.99	64.22±6.50	0.001
HC (cm)	101.21±9.78	80.57±8.56	0.001
WHR	0.84±0.08	0.799±0.06	0.001
TC (mg dL^{-1})	131.70±29.28	120.85±35.41	0.001
TG (mg dL^{-1})	140.13±35.52	116.18±20.85	0.001
HDL-c (mg dL^{-1})	48.13±8.59	50.49±8.90	0.156
LDL-c (mg dL^{-1})	83.26±8.50	77.25±7.39	0.001
VLDL-c (mg dL^{-1})	26.65±6.0	24.57±5.01	0.684
FBG (mg dL^{-1})	86.27±11.75	85.49±13.07	0.760

*Data are presented as Mean±SD and frequency (%), †p-value is significant for values less than 0.05, p-values represent the difference between groups, BMI: Body mass index, WC: Waist circumference, HC: Hip circumference, WHR: Waist-to-hip ratio, TC: Total cholesterol, TG: Triglycerides, HDL-c: High-density lipoprotein cholesterol, LDL-c: Low-density lipoprotein cholesterol

Table 2: Association between antioxidant enzymes and selected indicators

†Indicator	SOD	CAT	GPx
BMI	0.456**	0.186	0.020
WC	0.322*	0.253	0.067
HC	0.369**	0.137	-0.007
FBG	-0.180	0.146	-0.043
TC	-0.103	-0.098	0.115
TG	0.172	0.140	0.187
HDL-c	0.172	-0.204	0.166
LDL-c	0.330**	-0.012	0.130

†Values are presented as correlation coefficient (r), *p<0.05 and **p<0.001 are significant, p-values represent the difference between groups. BMI: Body mass index, WC: Waist circumference, HC: Hip circumference, FBG: Fasting blood glucose, TC: Total cholesterol, TG: Triglycerides, HDL-c: High density lipoprotein cholesterol, LDL-c: Low density lipoprotein cholesterol

Table 3: Association between MDA and selected indicators

†Indicator	MDA
BMI	0.531**
WC	0.453**
HC	0.487**
FBG	0.074
TC	0.233
TG	0.315*
HDL-c	0.036
LDL-c	0.378**
FBG	0.074
SOD	0.328**
CAT	0.051
GPx	0.251

†Data are presented as correlation coefficient (r), *p<0.05 and **p<0.001 are significant, p-values represent the difference between groups. BMI: Body mass index, WC: Waist circumference, HC: Hip circumference, FBG: Fasting blood glucose, TC: Total cholesterol, TG: Triglycerides, HDL-c: High-density lipoprotein cholesterol, LDL-c: Low-density lipoprotein cholesterol

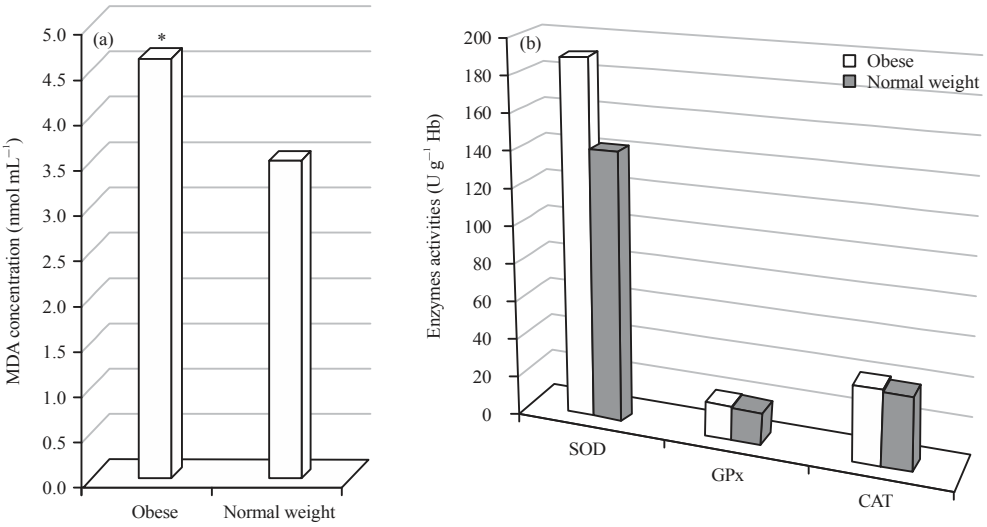

Fig. 1(a-b): (a) Mean activities of antioxidant enzymes in obese and normal weight students, (b) Mean MDA concentration, data are presented as Mean ± SD, *p-value is significant for values less than 0.05, p-values represent the difference between groups, MDA: Malondialdehyde, SOD: Superoxide dismutase, GPx: Glutathione peroxidase, CAT: Catalase

Risk factors that increase antioxidant enzyme activities: The findings of the present study demonstrate that the activity of the first enzyme in the antioxidant response, SOD was significantly higher in obese children and adolescents relative to their normal weight counterparts regardless of gender ($p<0.05$). Meanwhile, activity of GPx and CAT were not affected by increased BMI. A study of Tunisian children produced similar findings in that obesity was positively associated with increased SOD enzyme activity even at an early age and there was no significant difference between GPx and CAT activity in obese and normal weight children[9].

Mitochondria in white adipose tissue, particularly that from obese individuals are a main source of Reactive Oxygen Species (ROS) such as O_2^- and H_2O_2. The ROS generation is accompanied by an increase in NADPH oxidase expression and decreased expression of antioxidant enzymes. Moreover, adipocytes secrete inflammatory cytokines which are potent stimulators of ROS production by macrophages and monocytes. Elevated cytokine levels could also be responsible for increases in oxidative stress seen with obesity[6]. Tumor Necrosis Factor alpha (TNF-α) in particular may promote generation of O_2^- from oxygen by inhibiting the activity of photosynthetic carbon reduction and interaction of electrons with oxygen to generate O_2^-. Adipose tissue also secretes angiotensin II, which stimulates NADPH oxidase activity that is a major route for ROS production[4].

This study findings show significant positive associations between SOD activity and BMI ($r = 0.456$, $p<0.05$), WC ($r = 0.322$, $p<0.05$) and HC ($r = 0.369$, $p<0.001$). A similar significant positive correlation was seen between SOD and BMI ($r = 0.77$) and WC ($r = 0.632$) in obese Egyptian adolescents[17], but in a study of obese Tunisian children SOD activity correlated only with BMI ($r = 0.30$, $p<0.001$)[9]. Interestingly, this study found that abdominal fat enhanced both lipid peroxidation as manifested by increased MDA levels and SOD activity, but the mechanisms by which abdominal adiposity could induce increases in oxidative stress are unclear. One possible mechanism for this increase is that oxidative stress could be induced by low-grade systemic inflammation as characterized by higher C Reactive Protein (CRP) and interleukin-6 (IL-6) concentrations, which in turn lead to a low-grade inflammatory state that induces free radical production and subsequent increases in lipid peroxidation[18].

Risk factors associated with oxidative stress: The current study demonstrated that MDA concentrations were highly elevated in obese children and adolescents relative to normal weight student ($p<0.001$). The MDA levels were also positively associated with BMI ($r = 0.531$, $p<0.05$). In addition, SOD levels were positively associated with increased MDA, suggesting that in the Jordanian children examined here, higher amounts of oxidative stress could enhance the antioxidant enzyme response that serves as a defense mechanism against oxidative damage. This possibility is consistent with that suggested in an earlier report by Albuali[19].

Lipids react with ROS to produce lipid peroxides such as lipid hydroperoxide, which is hydrolyzed to a complex mixture

of compounds that includes aldehyde as the predominant molecule. Excess MDA production has toxic effects on antioxidant enzymes in that MDA can modify amino acid side chains and oxidize thiol groups in antioxidant enzymes; these modifications often result in partial or complete loss of activity[20]. Abnormal metabolism and metabolites in adipose tissue may generate and promote release of excessive amounts of proinflammatory and inflammatory cytokines and abnormal metabolism of other biochemical constituents could induce production and release of large amounts of O_2^-, •OH, H_2O_2 and other ROS that increase oxidative stress and lipid peroxidation[21,22].

CONCLUSION

Obesity-related increases in oxidative stress were observed even during childhood and adolescence. Increased levels of oxidative stress in obese children and adolescents were associated with increased amounts of MDA which reflects an imbalance between ROS production and antioxidant defense mechanisms. The SOD activity increased with increases in BMI, resulting in an enhanced antioxidant response. This study did not assess the eating habits or physical activity of the study subjects which could have important effects on antioxidant enzyme activity and oxidative stress in the obese individuals. Given the multifaceted nature of oxidative stress, future studies should consider additional parameters when quantifying levels of oxidative stress.

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