Amelioration of Oxidant-Antioxidant Stress in Morbid Obese Saudi Children by Carbohydrate-Restricted Diet

Waleed Albuali

*Assistant Professor,Department of Pediatrics, College of Medicine, King Faisal University, Al-Ahsaa, Kingdom of Saudi Arabia.

Abstract

Background: The effect of low carbohydrate diet on oxidant-antioxidant status in morbid uncomplicated obese children is very limited. **Aim:** The present study was designed to evaluate the activities of antioxidant enzymes and oxidant products in obese Saudi children and matched age and genders control subjects before and after low carbohydrate diet. **Subjects and Methods:** A prospective study has done on 105 Saudi children. They were classified into two groups; uncomplicated morbid obesity, and matched age control. All subjects underwent anthropometric measurements and estimation of the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GSSG-R), the concentrations of reduced glutathione (GSH), malondialdehyde (MDA), advanced oxidation protein products (AOPP) and oxidized low-density lipoproteins (ox LDL) in blood of obese and normal weight children before and after restriction carbohydrate diet. **Results:** The obese children had a significantly higher body mass index (BMI) and waist-to-hip ratio (WHR) compared with controls. The activities of enzymes and GSH levels were significantly reduced in obese children. The mean values of Ox LDL, MDA, and AOPP were significantly elevated in obese children compared to that of normal weight children. Oxidant-antioxidant status improved after 6 months of carbohydrate-restricted diet, which was associated with a reduction of BMI and WHR. **Conclusion:** Oxidant-antioxidant status is changed in morbid obese children and is returned to baseline levels after another 6 months of low carbohydrate diet which should be maintained over time in this age.

Keywords: Obese children; Body mass index; Malondialdehyde; Superoxide dismutase; Catalase; Glutathione peroxidase; Glutathione reductase; Reduced glutathione, Dietary-restriction of carbohydrates.

Introduction

Childhood obesity is the most prominent chronic problem among children in developed and developing countries which is associated with the risk of cardiovascular disease, dyslipidemia, atherosclerosis and other diseases in adulthood.[1,2] In 1998, *Berenson et al*[3], demonstrated that the signs of coronary heart disease, such as coronary artery fatty streaks, are observed in childhood and rapidly increase during adolescence particularly in those with elevated body mass index (BMI). It has been demonstrated that oxidative stress plays a central role in the diseases related to obesity.[4]

Oxidative stress results from an imbalance between the production of reactive oxygen species (ROS), such as superoxide ($O_2^{\cdot-}$) and hydroxyl ($^{\cdot}OH$) radicals, with antioxidants defenses, that leads to oxidative damage of lipids, proteins, and DNA and might be a major mechanism underlying obesity-related complications.[5]

The human body has developed several

Corresponding Author: Dr. WaleedAlbuali, Department of Pediatrics, College of Medicine, King Faisal University, Al-Ahsaa, Kingdom of Saudi Arabia. E-mail: walbuali@yahoo.com, wbuali@kfu.edu.sa

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mechanisms to protect biomolecules from the deleterious effects of ROS. These include the antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GSSG-R) and glutathione peroxidase (GSH-Px), as well as water and lipid-soluble antioxidants, such as glutathione (GSH), ascorbate (vitamin C), α-tocopherol (vitamin E), and β-carotene.[6] They either detoxify reactive oxygen species,convert superoxide radicals($O^{\bullet -}$) into H_2O_2 or metabolize peroxide organic molecules. Glutathione protects the body organs against oxidative stress of ROS either directly as an antioxidant or indirectly by maintaining other cellular antioxidants in a functional state.[7]

Several markers of oxidative stress have been proposed as being useful to investigate the relationship between severe obesity and oxidative stress. The whole-body oxidative stress is best reflected by systemic levels of lipid peroxidation, e.g. malondialdehyde (MDA) and oxidized LDL (ox LDL), which are considered the most reliable oxidative biomarkers.[8] The levels of MDA were significantly increased in obese children compared with non-obese children. Oxidized low density lipoprotein (ox LDL), another marker of oxidative stress, is associated with obesity.[9]

Proteins are recognized for oxidants by ROS which then may undergo structural and functional modifications leading to endothelial dysfunction. Advanced oxidation protein products (AOPPs) arenovel markers of increased oxidative stress, which has some advantages over other markers because of their relatively early formation, greater stability, ease of determination and reliability and also their longer life span (10) and are considered reliable markers to estimate the degree of oxidant-mediated protein damage.

In 2001, *St Jeor et al*[11], reported that a carbohydrate-restricted diet will adversely affect serum lipid concentrations. In addition, some previous studies demonstrated that healthy volunteers following a low-carbohydrate diet to lose weight.[12-14] Furthermore, *Samaha et al*[15], found that severely obese subjects with a high prevalence of diabetes or the metabolic syndrome lost more weight during six months on a carbohydrate-restricted diet than on a calorie and fat-restricted diet, with a relative improvement in insulin sensitivity and triglyceride levels.

Although the prevalence of morbid obesity and obesity related complications in children has greatly increased in the eastern region of Saudi Arabia, the data about the effect of low carbohydrate diet on oxidant-antioxidant status in those obese children are scanty.Therefore, the current study aimed to evaluate the activities of SOD, CAT, GSH-Px, and GSSG-R, as well as the levels of MDA, ox LDL, and AOPP in obese and normal weight children before and after low carbohydrate diet program.

Material sand Methods

Study Design

The investigations were carried out in obese children referred to Hospital Paediatric Clinic and attendees of the Polyclinic Center at King Faisal University in Al-Ahsaa, Saudi Arabia between August 2009 and April 2011. The present study comprised of105 children (5-12 years, mean age 8.7 ±1.8 years; boys n = 83;79.0 % & girls n = 22; 21.0 %). The children were free of endocrinological (e.g. hypothyroidism) or liver disorders, and genetic syndromes associated with obesity. Clinically they were stable without symptoms of any acute infections in order to avoid the possible influence of such conditions on the parameters examined. None of the children wassmoking. They had no undertakenweight-loss/obesity treatment in the last 3 months.

Study Population

Children were classified into two groups; severe obese and normal weight, based on BMI (kg m^2) using the International Obesity Task Force (IOTF) criteria (16). Group I included 75

children with uncomplicated morbid obesity of BMI e" 95th percentile or BMI e" 30 kg/m^2 or more, 55 of them completed the study, and group II included 50 children of the same age as normal control of BMI= 18.5-25kg/m^2 or < 85th percentile. BMI z-scores were calculated based on the United States Centre for Disease Control and Prevention reference data.[17]

Ethical Approval: The study was approved by the Ethical Committee of the University of King Faisal University.

Anthropometric Measurements

Anthropometric measurements followed the protocolsof the International Society for the Advancement of Kinanthropometry.[18] Height was measured with a wall-mounted stadiometer (SECA 770, Hamburg, Germany) in relaxed position and arms hanging freely and without shoesto the nearest 0.3 cm, with participants barefoot. Weight was measured using electronic digital scales (TANITA ultimate scale 2000 scales, Tanita Corporation, Tokyo, Japan) to the nearest 0.1 kg, with childrenwearing only a hospital gown and underwear. Measurements were taken by single technician to overcome inter-rater error.BMI was calculated as weight in kilograms divided by height in meters squared.

Calculation of waist-to-hip ratio (WHR): To calculate WHR, the waist circumference was measured at its smallestpoint between iliac crest and rib cage and the hip circumference at itslargest width over the greater trochanters. Blood pressure was measured using a mercurygravitymanometer with proper cuff size in standardconditions andambulatory blood pressure monitoring was carried out.[19]

Demographics/Background Information

Parents completed a questionnaire to collect information about house hold income, maternal education, child medications and any medical conditions and oral consent from all children.

Blood Sampling

Blood samples were freshly drawn from the vein of various children groups after an overnight fasting on heparin at in-patient Hospital Pediatric Clinic and Polyclinic Center and were immediately transferred to our laboratory at the College of Medicine, King Faisal University in an icebox. Blood samples were centrifuged at 4000 rpm at 4°C immediately and plasma was stored at -20°C until analysis. The 50 µl of RBC were taken and lysed with1.0 ml ice-coldwater and the clear lysate obtained afterspinning down the cell debris at 8500 g for 10 min at 4°C was used for the assays.

Carbohydrate-Restriction Diet

No specific exercise program was recommended. The parents restricted carbohydrate intake to 30 g per day or less for their children by providing vegetables and fruits with high ratios of fiber to carbohydrate.[20] No instruction on restricting total fat intake was provided and no vitamin preparation was recommended

Biochemical Analysis

The following laboratory procedures are applied before and after dietary intervention.

1. *Determination of Hemoglobin (Hb %):* Hb was estimated spectrophotometrically (Boeco™ S-20 Spectrophotometer, Hamburg, Germany) by using kit obtained from Biodiagnostic™ Cairo, Egypt according to the method of *Ranganathan and Gunasekaran.*[21] The values are expressed as g/dL.
2. *Estimation of Blood Glucose:* Blood glucose concentration was estimated spectrophotometrically (Boeco™ S-20 Spectrophotometer, Hamburg, Germany) through application of method described

by *Freund et al*,[22] by using enzymatic test kit (glucose oxidase) supplied by Biodiagnostic™, Cairo, Egypt. The results were expressed as mg/dL.

3. *Estimation of Total Serum Protein:* The total plasma proteins were estimated by using spectrophotometric (Boeco™ S-20 Spectrophotometer, Hamburg, Germany) method of *Flack and Woollen* (23). The results are expressed as g/dl

4. *Measurement of Concentrations of Oxidative Productsand Activities of Antioxidant enzymes:*

i. *Determination of Malondialdehyde:* Malondialdehyde (MDA) level, an end product of lipid peroxidation of erythrocytes, was assayed spectrophotometrically (Boeco™ S-20 Spectrophotometer, Hamburg, Germany) by using a diagnostic kit supplied by Biodiagnostic™, Cairo, Egypt, by using the method of *Stocks et al.*[24] The results were expressed as nmol/gHb.

ii. *Determination of Plasma Oxidized Low-Density Lipoproteins (oxLDL):* oxLDL level was estimated by using enzyme-linked immunosorbent assay (ELISA; Merocdia™, Inc., Winston-Salem, NC, USA)kit according to the method described by *Lehtimäki et al.*[25] The concentration of ox LDL is expressed in mg/g protein.

iii. *Advance Oxidation Protein Products (AOPP):* Determination of AOPP was based on spectrophotometric detection of chloramin T at 340 nm according to the method of *Witko-Sarsat et al.*[26] Concentration of AOPP is expressed in chloramine units (ìmol/g protein).

iv. *Superoxide Dismutase (SOD) Activity (SOD; EC 1.15.1.1)*: *Halliwell and Gutteridge* method[27] was used to estimate the total SOD activity spectrophotometrically (Boeco™ S-20 Spectrophotometer, Hamburg, Germany) in RBCs hemolysate by using test kit obtained from Spin React Biodiagnostic™, Cairo, Egypt. The results were expressed as U/g Hb.

v. Glutathione peroxidase (GSH-Px; EC 1.11.1.9) the activity of GSH-Px in erythrocytes was estimated spectrophotometrically (Boeco™ S-20 Spectrophotometer, Hamburg, Germany) by using the method described by *Paglia and Valantine*[28] by using a diagnostic kit provided by Biodiagnostic™, Cairo, Egypt. The results were expressed as mU/g Hb.

vi. Glutathione Reductase (GSSG-R; ECEC 1.6.4.2): Erythrocyte GSSG-R activity was determined spectrophotometrically (Boeco™ S-20 Spectrophotometer, Hamburg, Germany) by using a diagnostic kit provided by Biodiagnostic™, Cairo, Egypt as described by *Worthington and Rosemeye.*[29] The results were expressed as mU/g Hb.

vii. Catalase Activity (CAT; EC 1.11.1.6): CAT activity was measured spectrophotometrically (Boeco™ S-20 Spectrophotometer, Hamburg, Germany) using a standard CAT assay kit Biodiagnostic™, Cairo, Egypt, through following the decomposition rate of H_2O_2 at 240 nm according to the method of *Aebi.*[30] The results were expressed as U/g Hb.

viii.Reduced Glutathione (GSH): GSH, was assayed using the method of Anderson.[31] The results were expressed as mg/gHb

Statistical Analysis

Thedata were reported as mean ± standard deviation and analyzed with the SPSS 16.0.7 (SPSS™, Chicago, IL, USA) for Microsoft Windows XP™ (Redmond, WA, USA) statisticalsoftware package. Differences between thegroups were evaluated using the Student'sindependent-samples *t*-test (normally distributeddata) or Mann–Whitney U-test (non-normally distributeddata).Group comparison was performed byusing a one-

Table 1: Characteristics, Glucose and Hemoglobin Levels of Obese and Control Children Enrolled in the Study

Characteristic	Subjects		
	Normal weight (n = 55)	Obese before low CHO-diet (n = 50)	Obese after low CHO-diet (n = 50)
Gender			
Boys (No%)	40 (72.7)	40 (80)	
Girls (No%)	15 (27.3)	10 (20)	
Age in years			
(mean ± SD)	9.1 ± 4.3	8.9 ± 3.8	
t-test and P value		1.894†, 0.183*	
Residence			
Urban (No%)	17 (30.9)	19 (38.0)	
Rural (No%)	38 (69.1)	31 (62.0)	
Glucose (mg/dl)			
(mean ± SD)	87.4±9.4	88.2± 11.2	86.9 ± 11.2
t-test and P value[?]		2.036†, 0.089*	2.682†, 0.137*
t-test and P value[??]			2.715†, 0.182*
Hemoglobin (Hb; g %)			
(mean ± SD)	14.9±5.3	14.3±5.9	13.9±7.3
t-test and P value[?]		1.412†, 0.611*	1.959†, 0.734*
t-test and P value[??]			1.273†, 0.801*
Systolic blood pressure (mmHg)			
(mean ± SD)	116.9 ± 7.9	116.2 ± 8.8	115.5 ± 8.4
t-test and P value[?]		1.381†,0.880*	1.461†,0.860*
t-test and P value[??]			1.381†,0.890*
Diastolic blood pressure (mmHg)			
(mean ± SD)	79.5 ± 8.7	80.3 ± 9.1	78.9 ± 7.8
t-test and P value[?]		1.595†, 0.952*	1.595†, 0.973*
t-test and P value[??]			1.983†, 0.894*
Anthropometry:			
Height in cm	139.9 ±12.8	140.4±13.4	
mean ± SD			
t-test and P value		*0.034†, 1.094**	
Body Weight in Kg			
mean ± SD	59.2 ±8.4	147.9 ± 15.9	85.2 ± 9.2
t-test and P value[?]		*20.030†, 0.0001[a]*	*9.931†, 0.0001[a]*
t-test and P value[??]			*23.147†, 0.0001[b]*
Body mass index!(BMI)			
mean ± SD			21.5±9.4
t-test and P value[?]	17.9±3.6	39.8±10.8	11.451†, 0.0001[a]
t-test and P value[??]		28.072†, 0.0001[a]	21.706†, 0.0001[b]
BMI z-score		2.41 ± 0.12	0.09 ± 0.04
mean ± SD		18.197†, 0.0001[a]	6.849†, 0.0001[a]
t-test and P value[?]	0.05 ± 0.02		15.513†, 0.0001[a]
t-test and P value[??]			
Waist/hip ratio (WHR)			
(mean ± SD)	0.66± 0.06	0.89 ± 0.11	0.69 ± 0.07
t-test and P value[?]		*12.098†, 0.0001[a]*	*3.254†, 0.01[a]*
t-test and P value[??]			*12.254†, 0.0001[b]*

Values are presented in means ± SD, SD = standard deviation. !Body mass index = weight in kg/height in meter^2. †*t*-test for independent samples.*Non-significant values of obese group *vs* control, [a]significant values of obese before or after CHO-diet group *vs* control. [b]significant values of obese after CHO-diet group *vs* obese before CHO-diet group.

[I%]Obese before or after low CHO-diet *vs* control

[I%I%]Obese after low CHO-diet *vs* obese before low CHO-diet

Table 2: Erythrocytes Activities of Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GSH-Px), Glutathione Reductase (GSSG-R), and Reduced Glutathione Concentration (GSH)

Group	SOD (U/g Hb)	CAT (mg/g Hb)	GSH-Px (mg/g Hb)	GSSG-R (mg/g Hb)	GSH (mg/g Hb)
Normal weight					
(n)	(55)	(55)	(55)	(55)	(55)
Mean ± SD	218.9±37.3	69.8±12.2	56.3±9.8	64.8±10.6	54.3±9.7
Obese before low CHO-diet					
(n)	(50)	(50)	(50)	(50)	(50)
Mean ± SD	187.4± 18.4	48.3 ±9.3	37.3 ±8.6	32.9 ±7.6	33.3 ±8.2
t-test and P value	*12.32†, 0.001[a]*	*10.92†, 0.001[a]*	*8.19†, 0.001[a]*	*9.52†, 0.001[a]*	*6.12†, 0.001[a]*
Obese after low CHO-diet					
(n)	***(50)***	***(50)***	***(50)***	***(50)***	***(50)***
Mean + SD	204.6 ± 28.8	59.3 ± 9.3	48.1±10.4	52.5 ± 12.5	40.7±11.9
t-test and P value[?]	*13.12†, 0.0001[a]*	*11.02†, 0.0001[a]*	*14.19†, 0.0001[a]*	*14.36†, 0.0001[a]*	*15.72†, 0.0001[a]*
t-test and P value[??]	*16.53†, 0.0001[b]*	*14.68†, 0.0001[b]*	*19.04†, 0.0001[b]*	*24.63†, 0.0001[b]*	*19.18†, 0.0001[b]*

Values are presented in means ± SD, SD = standard deviation. †*t*-test for independent samples. [a]significant values of obese before or after CHO-diet group *vs* control. [b]significant values of obese after CHO-diet group *vs* obese before CHO-diet group.
[I%]Obese before or after low CHO-diet *vs* control
[I%I%]Obese after low CHO-diet *vs* obese before low CHO-diet

way analysis of variance (ANOVA). Differences was considered statistically significant at $p< 0.05$.

Results

Basic Characteristics

Clinical characteristics, anthropometric measurements, glucose and hemoglobin (Hb) of obese children and their controlare demonstrated in Table 1. A total of 105 children(normal weight = 50; obese = 55) with age ranged from 5-12 years (mean age8.7 ± 1.8 years; boys n = 80; 76.2% & girls n = 25; 23.8 %). Age, gender, height, blood pressure and levels of fasting glucose and Hb did not differ between groups either before or after low CHO-diet. Body weight, BMI, BMI z-score and WHR were significantly elevated among obese children before low CHO-diet compared with the healthy-weight group (Table 1). Furthermore, body weight, BMI, BMI z-score and WHR were significantly reduced in obese after 6 months low CHO-diet compared with the obese group before low CHO-diet but still different from the values of the control group. Obese children were resided more in the Rural region (Table 1).

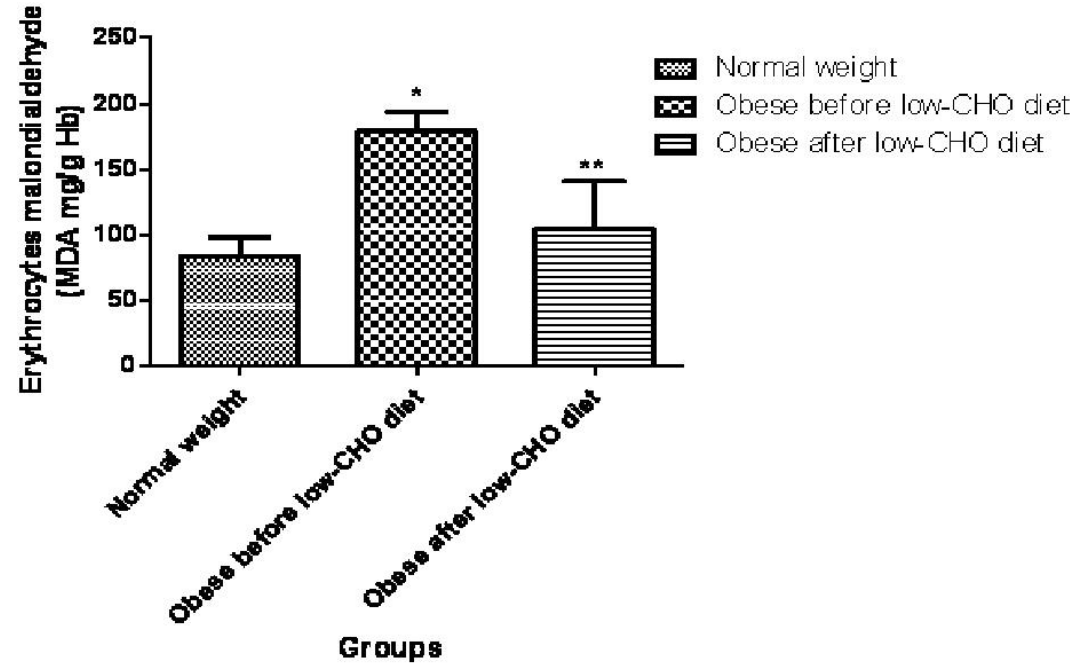
Oxidant-Antioxidant Markers Before Carbohydrate-Restriction Diet Program

The erythrocyte activities of SOD, CAT, GSH-Px and GSSG-R as well as the GSH concentration were registered in Table 2. The erythrocyte activities of SOD, CAT, GSH-Px and GSSG-R and erythrocyte GSH contents were significantly decreased in obese group before low carbohydrate diet compared to the corresponding values of the normal weight group (Table 2). The concentrations of MDA, OxLDL and AOPPwere shown in Fig-1; A, B, and C. MDA, OxLDL, and AOPP were significantly increased in obese children before low carbohydrate diet compared to the normal weight children.

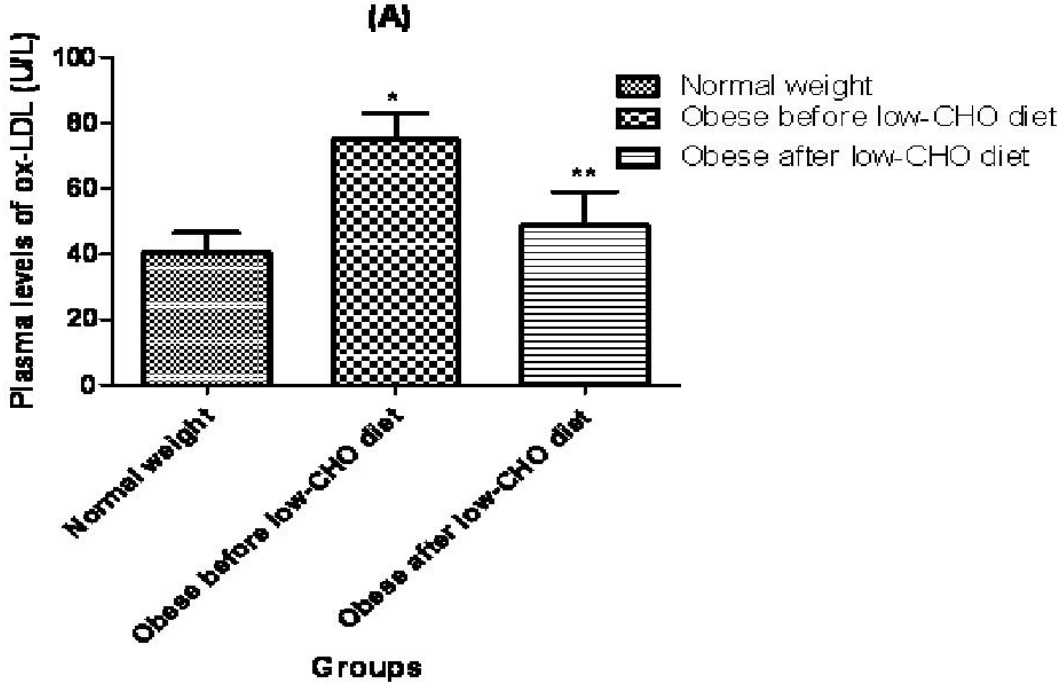
Oxidant-Antioxidant Markers After Carbohydrate-Restriction Diet Program

During the carbohydrate-restriction program, all children followed the prescribed low carbohydrate diet as they had a highvegetable and fruit intake. After 6 months low carbohydrate diet, obese children showed a significant increase in the activities of SOD,

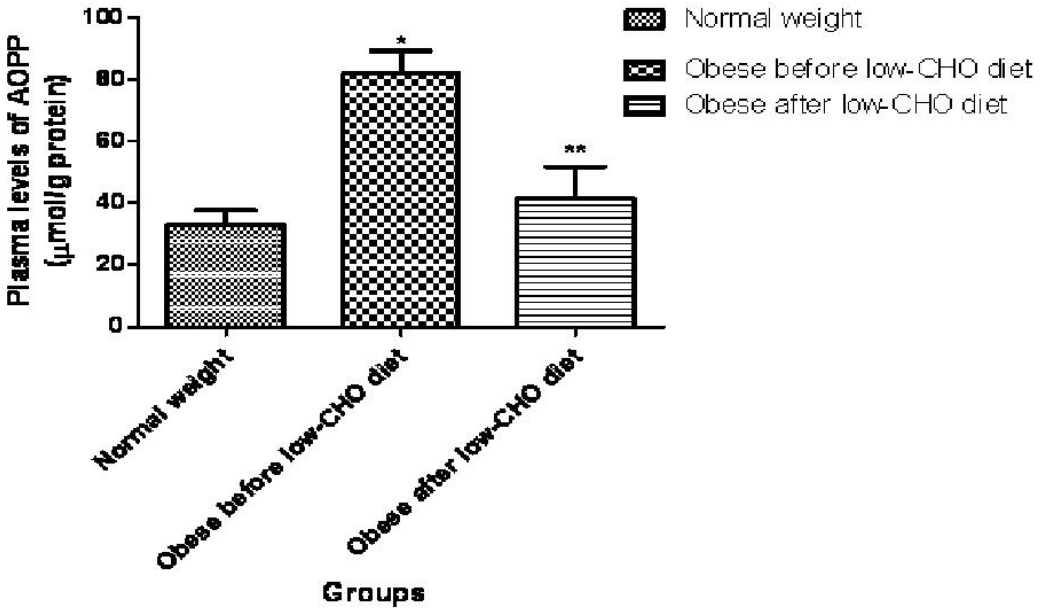
Fig 1: Oxidant Products in the blood of Normal Weight and Obese Children. Results are Expressed as Mean ± SD. (A) Erythrocyte Levels of Malondialdehyde (MDA mg/g Hb) in Normal Weight and Obese Children Before or After Low CHO Diets. (B) Plasma Levels of Oxidized Low-Density Lipoproteins (ox-LDL) in Normal Weight, and Obese Children Before or After Low CHO Diets. (C) Plasma levels of Advanced Oxidation Protein Products (AOPPs) in Normal Weight, and Obese Children Before or After Low CHO Diets. CHO = Carbohydrate



(A)



(B)



(C)

*Significantlyelevation of MDA, ox LDL and AOPP concentrations in obese children before low CHO diet *vs* control
**Significantly reduced of MDA, ox LDL and AOPP concentrations in obese after low CHO diet *vs* obese before low CHO diet but still high than the values of the control group

CAT, GSH-Px and GSSG-R as well as GSH content compared to the values of obese group before low carbohydrate diet (Table 2), but still significantly lower than values of the normal weight group. Furthermore, The concentrations of MDA, OxLDL, and AOPP were significantly reduced in obese children after low carbohydrate diet compared to the obese group beforelow carbohydrate but still also higher than the values of the healthy weight control groupt (Fig1; A, B and C).

Discussion

The present study estimated the glucose and hemoglobin levels to exclude the children who have either hyperglycemia or hyper- or hypoferremia because these conditions may affect the levels of the present parameters. In addition, the present study avoided the inherited obesity to exclude its affect the same parameters. Thus, the obtained data investigated the effect of the acquired fatness on the antioxidant status in children. The prevalence of obese children in the rural region may be attributed to the low maternal education (data not shown) and lack of physical activity. In addition, the major limitation of this study is therelatively small sample size because this might implicate that the results are not easily applicable to the whole populationof severely obese children.

Energy imbalances lead to the storage of excess energy in adipocytes, resulting in abnormalities of their functions, particularly mitochondria, and disrupted endoplasmic reticulum function, causing generation of excess ROS such as superoxide radicals ($O_2^{\cdot-}$) and H_2O_2 [32] Growing evidence indicates that mitochondria ofwhite adipose tissue (WAT) are the main site of ROS generation accompanied by augmented expression of NADPH oxidase and decreased expression of antioxidative enzymes.[33] This finding is confirmed by Mahadev et al[34], who reported that mRNA expression of NADPH oxidase increased in WAT of obese mice.

The present data showed that the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase

(GSSG-R) and catalase were depleted in obese children. The reduction of these enzymes may be attributed to the high production of ROS which may destroy these antioxidant enzymes.[35-37] In addition, the decrease of erythrocyte GSH level (Table II), which is an essential cofactor for GSH-Px, may lead to reduction of GSH-Px activity in obese children.[38,39] Furthermore, the reduced activities of these antioxidant enzymes in obese children may be attributed to the decreased expression of their mRNA. This finding is confirmed by the studies of *Li et al*[40], and *Furukawa et al*[41], which showed that the mRNA expression levels of antioxidant enzymes, such as SOD, CAT, GSH-Px, decreased in WAT of obese mice. The excess production of malondialdehyde (MDA) (Fig 1-A) have additional toxic effects for antioxidant enzymes. MDA maymodify the amino-acid side chains and oxidation of thiol groups of these enzymes, resulting in a partial or complete loss of their activities and functions.[42] The increased production of nitric oxide (NO) in obese children (data not shown) may act as an endogenous free radical scavengers that they react with superoxide radicals ($O_2^{\cdot\cdot}$) at a rate three times faster than the reaction of $O_2^{\cdot\cdot}$ with superoxide dismutase (SOD) (43), which may decrease SOD activity which occurs in our cases (Table 2). *Fang et al* [44], found that the oxidized low-density lipoproteins (Ox LDL) correlated negatively with SOD expression and they reported that the decreased activity of SOD may be attributed to excess production of ox-LDL which inhibits the expression of SOD.

GSH plays multiple roles in the cell including free radical scavenger as a primary antioxidant defense.[7]. The significant decrease of erythrocyte GSH content in obese children may be due to increase its turnoverinto its oxidized form (GSSG) through its detoxification of ROS and other peroxides to challenge the prevailing oxidative stress generated by ROS.[45] This is consistent with GSH function to scavenge oxidants by binding with them covalently.[46] Furthermore, the reduction in erythrocytes GSH content may be attributed to its usingin recycling of vitamin E and semi-hydroascorbic radicals and reduce oxidized molecules such as lipid hydroperoxides.[47] In addition, a decreasein the GSH level in the red cell may be resultingfrom depressed GSSG-R activity (Table II).[48]

The common approach in the measurementof oxidative stress is the determination of malondialdehyde (MDA), a product of lipid peroxidation. Thus, the excess production of ROS with insufficient antioxidant enzymes in obese children may have a serious adverse effect on cell membrane of RBCs resulting in lipid peroxidation enhancing production of MDA concentrations similar to our cases (Fig 1-A).[41] This finding is in agreement with the study of *Ustundag et al*[49], which showed the elevation of plasma MDA in smaller groups of obese children when compared with healthy controls.

The current study showed the increase level of oxidized LDL (ox-LDL), the second approach in the measurement of oxidative stress in obese children. Increased levels of ox LDL may be related to excess oxidative stress with lowered antioxidant defense.[50] This finding has been demonstrated in 1992 by *Parthasarathy et al*[51], who reported that obese children and adolescents have higher levels of ox LDL due to generation of ROS compared to normal-weight group

Advance oxidation protein products (AOPPs) are considered reliable markers to estimate the degree of oxidant-mediated protein damage. The observed increases in AOPPs levels in the present study suggest that proteins might be an important oxidative target of accumulation of oxidative stress in severe childhood obesity.[52] This argument has been confirmed by the study of *Atabeck et al*[53], which found that AOPP level was increased in obese children and adolescents.

The present data (Tables 1 & 2 and Fig 1) showed that the reduction of BMI and WHR after a 6-month low carbohydrate diet was associated with a reduction in oxidative stress, leading to modulate of antioxidant enzymes, GSH, MDA, ox LDL and AOPP nearly to those

of normal-weight children. This beneficial effect of low carbohydrate diet might be attributed to the suppressing generation of ROS by weight loss. These findings are supported by the studies in adults which found that the decrease in oxidative stress is associated with weight loss.[54,55]

Therefore, the present data showed that the persistent over nutrition might expose obese children to oxidative stress through excessive generation of ROS and the carbohydrate restriction diet might reduce this oxidative stress.[56,54]

Conclusion

The present data demonstrate a significant imbalanced between oxidative and antioxidative systems in obese children which is completely reversible with low carbohydrate diet and weight loss.In addition, there is a strong association between markersof oxidative stress, such as MDL, ox LDL and AOPP and both BMI and WHR.

Declaration of interest: The author reports no conflicts of interest. The author alone is also responsible for the content and writing of the paper.

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