

Overweight and Obesity—Induced Oxidative Stress in Children

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Objective To investigate whether overweight and obesity might cause oxidative stress and potential oxidative damage in overweight and obese children, and to explore its possible mechanism. **Methods** Eighty-five overweight and obese children (OOC), and eighty-five age-matched healthy children (HC) were recruited in this case-control study. The present study analyzed spectrophotometrically vitamin C (VC), vitamin E (VE), and β -carotene (β -CAR) in plasma, as well as the activities of superoxide dismutase (SOD), catalase (CAT), and the level of malondialdehyde (MDA) in erythrocytes. **Results** Compared with those of VC, VE, β -CAR, SOD, CAT and MDA in the HC group, the average values of VC, VE, β -CAR, SOD, and CAT in the OOC group were significantly decreased ($P < 0.001$), while the average value of MDA in the OOC group was significantly increased ($P < 0.001$). The regression analysis demonstrated that VC, VE, β -CAR, SOD, and CAT were negatively correlated ($P < 0.05-0.01$), and MDA was positively correlated with BMI ($P < 0.05$). Fitting to the model of multiple stepwise regression of BMI on VC, VE, β -CAR, SOD, CAT, and MDA in 85 OOC was $Y = 27.0041 + 0.2541_{MDA} - 2.1448_{\beta-CAR} - 0.0090_{CAT}$, where $F = 43.8088$, $P < 0.001$, $r = 0.7866$, $r^2 = 0.6187$, adjusted $r^2 = 0.6046$. The findings from the reliability analysis for VC, VE, β -CAR, SOD, CAT, and MDA used to reflect increased oxidative stress and potential oxidative damage in the OOC showed that the reliability coefficients (alpha, 6 items) = 0.7231, $P < 0.0001$, and that the standardized item alpha = 0.9207, $P < 0.0001$. **Conclusion** The present study suggests that there exists an increased oxidative stress in overweight and obese children.

Key words: Antioxidant; Antioxidative enzyme; Free radicals; Lipid Oxidation; Malondialdehyde; Obesity; Overweight; Oxidative stress

INTRODUCTION

Obesity is one of the most common nutritional disorders. However, little progress has been made in treatment and understanding of its causes. Obesity has been shown to impact negatively on health and be associated with increased risks of hypertension, type II diabetes mellitus, hyperlipidemia, cardiovascular and cerebrovascular diseases, degenerative joint disease, gall bladder disease, certain cancers, and psychosocial disability^[1-3]. These disorders and diseases may increase oxidative stress and cause potential oxidative damage due to production of excessive reactive oxygen species and free radicals^[4-6]. The prevalence of pediatric overweight and obesity has increased over the past few decades

in China. To date, there have been no reports on changes in oxidative stress in relation to body mass index (BMI) in Chinese overweight and obese children. A case-control study was therefore designed to confirm whether child overweight and obesity could cause an increased oxidative stress and to carry it out further to explore its possible mechanism.

MATERIALS AND METHODS

Study Design

In this study, 85 overweight and obese children (OOC) were recruited while 85 age-matched healthy children (HC) were chosen as the control. The present study measured plasma vitamin C (VC), vitamin E

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(VE), and β -carotene (β -CAR) as well as the activities of superoxide dismutase (SOD), catalase (CAT), and the level of malodialdehyde (MDA) in erythrocytes. In addition, the differences in the average values of above biochemical parameters between the OOC and the HC groups, the partial coefficient of correlation between the BMI and the said each parameter in 85 OOC, the fitting to the model of multiple stepwise regression of BMI on the said parameters in 85 OOC, and the reliability analysis for the said parameters were computed.

Subjects

OOC Selection Sixty-eight overweight children (BMI 25.03 to 29.88) and 17 obese children (BMI 30.26 to 34.81) were randomly sampled by the program of "select cases-random sample of cases" in "SPSS 11.0 for Windows" from 112 OOC confirmed by the diagnostic criteria^[1] and the inclusion criteria^[1] in the Second Affiliated Hospital, College of Medicine, Zhejiang University, China. There were 47 males and 38 females aged 5-15 years old in total. Duration for being overweight or obesity ranged from 1 to 11 years. Their hemoglobin, serum albumin, systolic blood pressure, and diastolic blood pressure were from 126.9 to 144.5 g/L, 35.14 to 46.36 g/L, 68 to 109 mmHg, and 45 to 70 mmHg, respectively. Agreed by their legal guardians, the above children

were all volunteers in this study.

HC Slection Eighty-five age-matched healthy children were randomly sampled by the said same program in "SPSS 11.0 for Windows" from 120 HC confirmed by the diagnostic criteria^[1] and the inclusion criteria^[1] in the said same hospital. There were 43 males and 42 females aged 5-15 years old with BMI ranged from 18.91 to 24.50. Similarly, their hemoglobin, serum albumin, systolic blood pressure, and diastolic blood pressure were measured and found to be 126.8-145.5 g/L, 35.19-46.07 g/L, 68-110 mmHg and 47-70 mmHg, respectively (Table 1).

Significant differences were found in average values of hemoglobin, serum albumin, systolic blood pressure, and diastolic blood pressure, as well as fasting levels of serum glucose, cholesterol, triglycerides, alanine aminotransferase, gamma-glutamyl transpeptidase, alkaline phosphatase between OOC group and HC group^[1] by independent-samples *t* test. However, no significant differences in education level, residence region, daily diet (food and drink), eating behavior, quantity and quality of dietary intakes, family history of obesity, and psychosocial factors were detected between the two groups^[1] (Table 1).

For their parents, there were no significant differences in average values of age, recent weight

TABLE 1

The Demographic Data and Some Other Data in the OOC Group and the HC Group

Item	OOC (n=85)	HC (n=85)	Levene's Test for Equality of Variances	Statistical Analysis
Age (year)	5-15* (9.35±2.72)**	5-15* (9.49±2.68)**	F = 0.155 P = 0.695	t = 0.348† P = 0.729
Gender	M = 47 F = 38	M = 43 F = 42	-	$\chi^2 = 0.378^\ddagger$ P = 0.645
Hemoglobin (g/L)	126.9-144.5* (138.8±5.1)**	126.8-145.5* (139.4±4.3)**	F = 2.505 P = 0.115	t = 0.714† P = 0.476
Albumin (g/L)	35.14-46.36* (42.09±2.24)**	35.19-46.07* (41.90±2.14)**	F = 0.073 P = 0.787	t = 0.561† P = 0.576
Systolic Blood Pressure (mmHg)	68-109* (91.5±9.2)**	68-110* (91.1±9.0)**	F = 0.046 P = 0.830	t = 0.262† P = 0.794
Diastolic Blood Pressure (mmHg)	45-70* (59.5±5.5)**	47-70* (59.0±5.8)**	F = 0.178 P = 0.673	t = 0.638† P = 0.524
Body Mass Index				
Overweight (n=68)	25.03-29.88* (27.06±1.47)**	-	-	-
Obesity (n=17)	30.26-34.81* (32.07±1.36)**	-	-	-
Total (n=85)	25.03-34.81* (28.06±2.48)**	18.91-24.50* (22.94±1.25)**	-	-
Course of Overweight and Obesity (year)	1-11* (5.3±2.8)**	-	-	-
Smoking History	No	No	-	-
Abusing Alcohol History	No	No	-	-

Note. * Range, ** Mean \pm SD ($\bar{x} \pm s$), † Independent Samples T Test, and ‡ Chi Square Test (Exact Sig.).

changes, occupational history, annual earning, education level, residence region, eating and exercise behavior, cigarette and alcohol use, and psychosocial factors between the two groups^[1].

The children with disorders associated with brain, heart, lungs, liver, kidneys, and other organs as well as blood, circulatory, respiratory system, and other systems were all excluded by routine blood, urine and stool examinations as well as radiographs, cardiogram, and other necessary examinations. The children with hypertension, hyperlipidemia, skin disorders, thromboembolic disorders, digestive tract diseases, acute or chronic bronchitis, asthma, autoimmune disease, diabetes, atherosclerosis, endocrine abnormalities, hypothyroidism, Cushing's syndrome, proteinuria, fatty liver disease, inflammation, tumors or/cancers, and undernutrition, malnutrition and other nutritional diseases were also excluded. In addition, all OOC and HC children recruited for the study had no psychosocial disability, smoking history, and excessive drinking history. None of the above recruited children had taken any antioxidant supplements such as VC, VE, β -carotene, ginkgo biloba, and tea polyphenols at least one month before the study.

Methods for Blood Assays

Collection and pretreatment of blood samples

Fasting venous blood samples were collected from all the children in the morning. Heparin sodium was added as an anticoagulant. Plasma and erythrocytes were immediately separated and stored at -50°C immediately. The blood samples did not undergo any hemolysis^[7-8].

Measurement of plasma VC Trichloroacetic acid solution was used to precipitate proteins in plasma and to extract VC from plasma. The VC in the extract solution reduced Fe^{3+} in the ferric trichloride solution to Fe^{2+} . Fe^{2+} reacted with ferrozine to form a colored end product that was measured using a spectrophotometer at 563 nm and the value was expressed as $\mu\text{mol/L}$ ^[7-8].

Measurement of plasma VE Absolute ethanol was used to precipitate proteins in plasma and to extract VE from plasma. The VE in the extract solution reduced Fe^{3+} in the ferric trichloride solution to Fe^{2+} . Fe^{2+} reacted with ferrozine to form a colored end product that was analyzed in a spectrophotometer at 563 nm and the value was expressed as $\mu\text{mol/L}$ ^[7-8].

Measurement of plasma β -CAR A mixture solution of ethanol and petroleum ether (1:1) was used to extract β -CAR from plasma. The extract solution containing β -CAR was analyzed in a spectrophotometer at 440 nm and the value was

expressed as $\mu\text{mol/L}$ ^[7-8].

Measurement of erythrocyte SOD activity Inhibition on pyrogallol auto-oxidation was used to determine erythrocyte SOD activity in a spectrophotometer at 420 nm and the SOD activity was expressed as $\text{U/g}\cdot\text{Hb}$ ^[7-8].

Measurement of erythrocyte CAT activity Coloration formation of hydrogen peroxide and acetic acid-potassium dichromate was used to determine erythrocyte CAT activity in a spectrophotometer at 570 nm and the activity was expressed as $\text{K/g}\cdot\text{Hb}$ ^[7-8].

Measurement of erythrocyte MDA level Production of thiobarbituric acid reactive substances (TBARS) was used to determine erythrocyte MDA level in a spectrophotometer at 532 nm and the value was expressed as $\text{nmol/g}\cdot\text{Hb}$ ^[7-8].

All analytical-graded reagents including in VC, VE, β -CAR, 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazinedisulfonic acid disodium salt (ferrozine), 1,2,3-trihydroxybenzene (pyrogallol), 1,1,3,3-tetraethoxypropane, 2-thiobarbituric acid (TBA), Cu \cdot Zn-superoxide dismutase, and catalase, were purchased from SIGMA[®] Chemical Company, USA. Other analytical reagents were purchased locally in China. Fresh quadruply distilled water was prepared with a quartz glass distilling apparatus. All tests were conducted using a Hewlett Packard 8453-spectrophotometer.

Statistical Analysis

All experimental data in this study were statistically analyzed with SPSS 11.0 for Windows statistic software (Serial number: 3805638; License code: 30376 40608 78517 08046 24431 4009) using a Compaq Pentium IV/2.4 GHz computer. The experimental parameters in this study presented normal distributions by Kolmogorov-Smirnov Z test, and were expressed as $\bar{x} \pm s$ and 95% confidence interval (95% CI). Hypothesis testing methods included independent-samples *t* test, Pearson chi-square test (χ^2 test), multiple correlation analysis, multiple stepwise regression analysis, and reliability analysis. In the statistical analysis, the level of hypothesis testing (α) was ≤ 0.05 in order to avoid false positives (type I error), and the power of hypothesis testing (*power*) was ≥ 0.85 to avoid false negatives (type II error)^[7-8].

RESULTS

Comparison Between the Average Values of VC, VE, β -CAR, SOD, CAT, and MDA ($\bar{x} \pm s$) in the OOC Group and the HC Group

Compared with those in the HC group, the

average values of plasma VC, VE, and β -CAR as well as erythrocyte SOD, and CAT in the OOC group were significantly decreased ($P<0.001$), while erythrocyte MDA in the OOC group was significantly increased ($P<0.001$) (Table 2).

95% Confidence Interval of the Average Values of VC, VE, β -CAR, SOD, CAT, and MDA ($\bar{x} \pm s$) in the OOC Group and the HC Group

The upper limits of 95% confidence interval (95% CI) of the average values of VC, VE, β -CAR, SOD, and CAT in the OOC group were less than the lower limits of 95% CI of those in the HC group. The

lower limit of 95% CI of the average value of MDA in the OOC group was greater than the upper limit of 95% CI of that in the HC group (Table 2).

Partial Coefficient of Correlation Between BMI and VC, VE, β -CAR, SOD, CAT and MDA in OOC Group

The partial coefficient of correlation of BMI with VC, VE, β -CAR, SOD, CAT, and MDA in 85 OOC while adjusting the age and the course of disease suggested that BMI was negatively correlated with the values of VC, VE, β -CAR, SOD, and CAT ($P<0.05-0.01$), while it was positively correlated with the value of MDA ($P<0.05$) (Table 3).

TABLE 2

Comparison Between the Average Values ($\bar{x} \pm s$) of VC, VE, β -CAR, SOD, CAT, and MDA in the OOC Group and the HC Group, and Their 95% CI

Group	n	Plasma			Erythrocytes		
		VC(μ mol/L)	VE(μ mol/L)	β -CAR(μ mol/L)	SOD(U/g-Hb)	CAT(K/g-Hb)	MDA(nmol/g-Hb)
OOC	85	45.48 \pm 11.57 (42.99–47.98)	20.36 \pm 4.69 (19.34–21.37)	1.46 \pm 0.37 (1.38–1.54)	1895 \pm 148 (1863–1927)	255.8 \pm 66.2 (241.6–270.1)	25.53 \pm 4.76 (24.50–26.56)
HC	85	56.34 \pm 13.95 (53.34–59.36)	26.80 \pm 4.62 (25.80–27.80)	1.75 \pm 0.51 (1.64–1.86)	2165 \pm 204 (2121–2209)	304.9 \pm 79.9 (287.6–322.1)	21.66 \pm 4.49 (20.69–22.63)
<i>F</i> *		2.064	0.544	3.549	2.820	1.917	3.104
<i>P</i>		0.153	0.462	0.061	0.095	0.168	0.080
<i>t</i> **		5.524	9.023	4.201	9.886	4.356	5.452
<i>P</i>		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Note. *Levene's Test for Equality of Variances. **Independent-Samples T Test. Figures in Parentheses are 95% CI.

TABLE 3

Partial Coefficient of Correlation Between BMI and VC, VE, β -CAR, SOD, CAT, and MDA in Overweight and Obese Children

Variables Correlated	n	Variables Adjusted	r	P
BMI and VC	85	Age and Course	-0.2882	0.008
BMI and VE	85	Age and Course	-0.3246	0.003
BMI and β -CAR	85	Age and Course	-0.3077	0.005
BMI and SOD	85	Age and Course	-0.3316	0.002
BMI and CAT	85	Age and Course	-0.2704	0.013
BMI and MDA	85	Age and Course	0.2609	0.017

Fitting to the Model of Multiple Stepwise Regression of BMI on VC, VE, β -CAR, SOD, CAT, and MDA in OOC

Regarding the value of BMI in each overweight or obese child as the dependent variable Y, and the values of VC, VE, β -CAR, SOD, CAT, and MDA as the independent variable X₁, X₂, X₃, X₄, X₅, and X₆, respectively, the fitting to the model of multiple stepwise regression of BMI on VC, VE, β -CAR, SOD, CAT, and MDA in 85 OOC was Y = 27.0041 +

0.2541MDA - 2.1448 β -CAR - 0.0090CAT, F = 43.8088, P<0.0001, r=0.7866, r²=0.6187, adjusted r²=0.6046, P<0.0001 (Table 4).

Matrix of Coefficients of Correlations of BMI and VC, VE, β -CAR, SOD, CAT, and MDA in OOC

The matrix of coefficients of correlations of BMI and VC, VE, β -CAR, SOD, CAT, and MDA in 85 OOC suggested that there were significant differences in pairwise comparison of the correlations

of BMI and VC, VE, β -CAR, SOD, CAT, and MDA ($P<0.05-0.001$) (Table 5).

TABLE 4

Fitting to the Model of Multiple Stepwise Regression of Body Mass Index on VC, VE, β -CAR, SOD, CAT, and MDA in 85 Overweight and Obese Children

Variable	Partial Coefficients of Regression	<i>t</i>	<i>P</i>	Partial Coefficient of Correlation		ANOVA	<i>R</i> value
				<i>r</i>	<i>P</i>		
Constant	27.0041	16.2118	<0.001	–	–		<i>r</i> = 0.7866
MDA	0.2541	6.4005	<0.001	0.5796	<0.001	<i>F</i> =43.8088	<i>r</i> ² =0.6187
β -CAR	-2.1448	4.3340	<0.001	-0.4339	<0.001	<i>P</i> <0.0001	adjusted <i>r</i> ² = 0.6046
CAT	-0.0090	3.2692	0.002	-0.3414	<0.001		<i>P</i> < 0.0001

Note. Dependent variable: Body mass index.

TABLE 5

Matrix of Coefficients of Correlations of BMI and VC, VE, β -CAR, SOD, CAT, and MDA in 85 Overweight and Obese Children

	BMI	VC	VE	β -CAR	SOD	CAT	MDA
BMI	1.000						
VC	-0.404**	1.000					
VE	-0.411**	0.924**	1.000				
β -CAR	-0.544**	0.176	0.139	1.000			
SOD	-0.487**	0.976**	0.889**	0.282**	1.000		
CAT	-0.474**	0.905**	0.897**	0.226*	0.890**	1.000	
MDA	0.679**	-0.310**	-0.331**	-0.349**	-0.351**	-0.331**	1.000

Note. * $P<0.05$, ** $P<0.01$.

Reliability Analysis for the VC, VE, β -CAR, SOD, CAT, and MDA that Were Used to Reflect Increased Oxidative Stress in OOC Group

The findings from the reliability analysis for the levels of VC, VE, and β -CAR in plasma as well as the activities of SOD, and CAT in erythrocytes, and the level of MDA in erythrocytes, by which were used to reflect increased oxidative stress and potential oxidative damage in the overweight and obese children were that the reliability coefficients' alpha (6 items) was 0.7231, $P<0.0001$, and that the standardized item alpha was 0.9207, $P<0.0001$.

DISCUSSION

VC, VE, β -CAR, SOD, and CAT are the most important free radical scavengers in humans, and play important roles in removal of excessive superoxide anion radical (O_2^-), hydroxyl radical ($\cdot OH$), singlet

oxygen (1O_2), hydrogen peroxide (H_2O_2), and other free radicals in humans^[9-19]. MDA and conjugated dienes are the products of peroxidative reactions (auto-oxidation) of lipids exposed to oxygen^[9-19]. Decomposition of lipid peroxides leads an increased MDA level and a decreased level of antioxidants, thus resulting in increased oxidative stress and potential oxidative damage^[9-19].

The findings in this study showed that there existed an imbalance between oxidation and antioxidation, an increased oxidative stress and potential oxidative damage in OOC group. To date, there has been neither report on increased oxidative stress, nor report about the partial correlation between the BMI and VC, VE, β -CAR, SOD, CAT, and MDA. The present study is the first report to examine obesity-induced oxidative stress in Chinese children. In addition, multiple stepwise regression among the BMI and each oxidative stress indexes, and the reliability analysis on the BMI and each oxidative indexes in OOC group were also conducted.

Obesity may be a low-grade systemic

inflammatory disease^[20-21]. In overweight and obese children and adults, the abnormal metabolism and metabolites in adipose tissue may generate and release excessive proinflammatory and inflammatory cytokines, such as interleukins (IL), notably IL-1beta, IL-6, IL-8, IL-12, and IL-18^[22-23], and tumor necrosis factor-alpha (TNF-alpha)^[22-23], and abnormal metabolism of other biochemical constituents, such as p53^[24-25], cytochrome P-450 and NADPH-cytochrome P-450^[26-27], C-reactive protein (CRP)^[20,28], xanthine^[29-30], adiponectin^[28,31], and leptin^[20,31], which could induce and release a large amount of O₂⁻, ·OH, ¹O₂, H₂O₂, and other ROS^[14,17,32-35]. Excessive O₂⁻, ·OH, ¹O₂, H₂O₂, and other ROS could attack directly DNA, damaging DNA, inhibiting or depressing DNA replication, and could also inactivate strongly the active sites and active groups of VC, VE, β-CAR, SOD, and CAT^[9-19]. Excessive free radicals and ROS could yet cause oxidative decomposition and peroxidative modification of many organic compounds in OOC, further deactivating antioxidants and antioxidative enzymes^[9-19]. As a consequence, the levels of plasma VC, VE, and β-CAR as well as the activities of erythrocyte SOD and CAT in the OOC were significantly decreased. At the same time, free radicals and ROS could accelerate and aggravate the lipid peroxidative reactions of polyunsaturated fatty acids, unsaturated phospholipids, glycolipids, cholesterol, other lipids, and lipid-contained organic compounds in blood, tissues, and cellular membranes in the OOC, leading to a significantly increased MDA level in the OOC^[7-11,13,16-19]. Additionally, the decreased VE level could *per se* result in significantly increased MDA level^[7-11,13,16-19].

The above abnormal biochemical and biophysical changes may not only cause oxidative stress and potential oxidative damage, but also reduce blood flow in hemodynamics, and decrease fluidity, viscoelasticity and deformability of erythrocytic membranes and other cellular membranes. This oxidative stress associated with overweight and obesity is most likely related to cardiovascular and cerebrovascular diseases^[36].

In this study, the partial coefficient of correlation while adjusting the age and the course of disease was used to compute the correlation between BMI and VC, VE, β-CAR, SOD, CAT, and MDA in 85 OOC^[17-19,36]. The findings from the partial coefficient of correlation suggested that the values of above biochemical substances and enzymes were closely related to the BMI in the OOC, and that with increased BMI, the values of VC, VE, β-CAR, SOD,

and CAT were gradually decreased, while the value of MDA was gradually increased. In other words, the higher was a child's BMI, the severer could be the increased oxidative stress and potential oxidative damage.

In this study, the fitting to the model of multiple stepwise regression of BMI on VC, VE, β-CAR, SOD, CAT, and MDA in 85 OOC suggested that there existed a closest correlation between the BMI and MDA, β-CAR, and CAT in the OOC. In this model, $r^2=0.6187$, and adjusted $r^2=0.6046$, such results suggested that in the childhood overweight and obesity the impact of malondialdehyde, β-carotene, and catalase on obesity was just about 60%, while about 40% was likely related to other factors dealing with both increased oxidative stress and obesity in children.

In conclusion, the findings in the present study suggest that overweight and obesity can cause increased oxidative stress and potential oxidative damage in overweight and obese children. It remains unexplored if weight loss may be a safe method for down-regulating the inflammatory state and decreasing oxidative stress and oxidative damage in overweight and obese children.

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