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Antioxidant Status and the Extent of Health Risks in Obese Korean Children

WonMyo Lee, EulSang Kim, Aewha Ha[1§], Ximena Urrutia-Rojas[1]

Department of Food Science and Nutrition, Dankook University, Seoul 140-714, Korea.

[1]Department of Social and Behavioral Sciences, University of North Texas Health Science Center, Fort Worth, Texas, USA

Objective : The purpose of this study was to determine serum antioxidant nutrients and the relationship between serum antioxidants and risks of chronic diseases in obese Korean children.
Methods : Normal weight Korean school children (n=170), mean age of 11.5±1.5, and obese (body fat mass > 28%) children (n=176), mean age of 11.0±1.8, were recruited. Fat mass (%) was determined by Bioelectrical Impedance (BEI), and body mass index (BMI) was calculated. Fasting blood was collected to measure serum antioxidant nutrients, vitamin A, vitamin E and zinc. Serum lipid profiles including total cholesterol (TC), high density cholesterol (HDL) and triglyceride (TG), and blood glucose, glutamic oxaloacetic transaminase (GOT), and glutamic pyruvic transaminase (GPT) were also determined. Differences in serum blood measurements between obese and normal children were assessed by independent t test. Pearson's correlation analysis was used to determine the relationship between variables.
Results : Blood glucose, GPT, total cholesterol, and triglycerides concentrations were significantly higher among obese boys, compared to normal boys ($p<0.05$). Significantly lower concentrations of serum vitamin E, after adjustment for TG and TC, was shown in obese boys (0.26 mg/mg) and obese girls (0.31 mg/mg), compared to normal boys (0.36 mg/mg) and girls (0.38 mg/mg) ($p<0.05$). Fat mass (%) was negatively correlated with serum vitamin A and vitamin E.
Conclusion : Obese Korean children showed insufficient serum vitamin E concentration and increased risk for diabetes, atherosclerosis, and liver disease. Since lower vitamin E concentration was negatively correlated with atherogenic index, improved vitamin E status in children may decrease the risk of atherosclerosis later in life.

Key words : Child obesity, Vitamin E, Vitamin A, Zinc, Blood glucose, GOP, Lipids

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INTRODUCTION

Overweight and obesity rates in Korean children are increasing. During the last 18 years (1979-1996), obesity rates in Korean children have increased; 6 times for boys and 4.7 times for girls.[1)] Fifty percent of all obese children will probably become obese adults.[2)] Childhood obesity leads to the early development of cardiovascular disease and diabetes.[3-7)] Children with obesity had higher rate of abnormal liver functions.[8-9)] Baldrige *et al.*'s study[8)] reported that obese children were more likely to have a fatty inflamed liver. Strauss *et al.*[9)] reported that overweight and obese adolescents had abnormal levels of serum ALT (alanine aminotransferase).

Antioxidants, such as retinol, beta-carotene, and vitamin E, have an important impact on the incidence of chronic diseases. Studies have shown that low antioxidant intake or low blood levels of antioxidants is related to a high incidence of chronic diseases such as cancer, heart disease, and diabetes.[10-16)] Subnormal blood levels of antioxidants have been reported in obese children,[17-20)] and low antioxidants in obese children have been reported to be associated with hyperinsulinemia,[20)] altered lipid metabolism,[17)] and altered liver functions.[9)]

Zinc is a cofactor of antioxidant enzyme, superoxide dismutase (SOD).[21)] Zinc is also an important nutrient that influences the thermoregulation in obese individual.[22)] Studies have been reported low blood zinc concentrations in obese adults. Some studies also reported that low zinc levels in the obese might be related with low secretion of leptin. However, inconsistent results, depending on human or animal studies, are present[23-27)] and limited information on blood zinc

[§] To whom correspondence should be addressed.
(E-mail : aewhaha@hanmail.net)

and obese children is existed.[28-30)]

Therefore, the purpose of this study was to determine whether obese children have decreased antioxidant nutrients including zinc and whether decreased antioxidant nutrients are associated with health risk factors of diabetes, arthrosclerosis, and liver disease.

METHODS

1. Subjects

The subjects of this study were 170 normal and 176 obese school children in Seoul, Korea, and surrounding Provinces. Obese children were selected from "Camps for Weight Control in Children" which were held during the summer of 1998. Age and sex-matched normal children were selected from 2 elementary schools, from July 1998 to December 1998, based on their school health records. A letter of invitation describing the project, an informed consent form, and a questionnaire were sent to the children's families three weeks prior to the study. Informed consent was obtained from the parent(s) of each child. The research project was approved by the Dankook University, Institutional Review Board, Seoul, Korea, in the summer of 1998.

2. Measurements

Weight was measured in kilograms using a digital electronic weighing scale, with the child wearing light clothes and no shoes. Height was measured in centimeters to the nearest 0.1cm using a portable stadiometer, with the child standing in a straight position. Bioelectrical Impedance (GLF-891 DX, Gilu Trading Co., Seoul, Korea) was used for screening body fat mass (%), as obesity diagnosis, in children.[31)] Children were asked to remove all metallic objects, such as necklace, watch, bracelets, rings, and hairpins before measurements. Children were then asked to lie on their back, with neither arms nor thighs touching. The children's skin on the top of the hand and wrist, and the top of the foot and ankle were cleaned with alcohol and 4 electric rods were attached to the hand, wrist, foot, and ankle. After turning the device on, the fat mass (%) of each child was recorded. Children, with fat body mass 28% or higher, were classified as obese.[32,33)] BMI (weight in kilograms over height, in meters, squared) was also calculated by using height and weight.

3. Blood Collection and Analysis

Children were required to fast at least 10 hours. After confirmation of fasting status, the school nurse drew blood between the hours of 9:00 a.m. and 11:00 a.m. Serum was separated from whole blood immediately and stored for further analysis.

Blood glucose, glutamic oxaloacetic transaminase (GOT), and glutamic pyruvic transaminase (GPT) were analyzed by VITROS 950 automatic analyzer. Serum concentrations of total cholesterol (TC) and triglyceride (TG) were analyzed by 700 XRN Analyzer (Kodak Edatachron). High density lipoprotein (HDL) was determined by general enzyme analyzing method after precipitation with $MgCl_2$ and dextran sulfate.[34)] Atherogenic Index (AI) was calculated by (total cholesterol − HDL)/HDL. Low-density lipoprotein (LDL) cholesterol was calculated by the formula, cholesterol − (triglyceride/5+HDL).[35)]

Serum concentrations of vitamin A and vitamin E were determined by the modification of Quaife and Dju's study.[36)] Serum (500 μml) was mixed with ethanol (500 μml). Hexane was added and the mixture was centrifuged for 10 minutes, at 12000 rpm, 4 ℃. The supernatant of hexane layer was collected, dried under nitrogen gas, and resolved again into its mobile gas phase. Each sample, 20 μl, was injected into the HPLC system (High Performance Liquid Chromatography, 2800 BIO-RAL, USA). In this HPLC system, reversed column (CR-18 (10 μm), 250×4 mm) (Supelco, USA) was used. Alpha-tocopheryl acetate and retinyl acetate were used as the internal standards. Flow rate was 1.5 ml/min, mobile phase consisted of 95% methanol/5% distilled water, and the wavelength of UV detector was 290 nm.

Serum zinc was analyzed by the modification of Rosado *et al*'s study.[37)] Each of the serum control and standard samples, 500 μml each, were mixed with 2 mL of distilled water. The absorbency was determined at 214 nm by using Flame Atomic Absorbance Spectrometer (AAS) (PERKIN ELMER 2380, USA). The quantification of zinc was calculated by using the zinc standard curve.

4. Data Analysis

Means and standard deviations were used in the descriptive analysis. Differences were regarded as statistically significant at values of $p<0.05$. Differences in serum vitamin levels between obese and normal children were assessed by independent t test. Pearson's correlation analysis was used to determine relationships between variables. The statistical analysis was performed with SPSS computer software (Chicago, USA).

RESULTS

Anthropometric and biochemical measurements of Korean children in the study are shown in Table 1. Age distribution of the subjects ranged from 10 to 12 years of age. The mean heights of two groups were similar, 147.8±7.2 cm in the obese and 144.8±13.0 cm in the normal. The mean body weight was significantly higher in obese children, 53.4±11.2, than in normal children, 37.4±13.0, ($p<0.05$). The mean BMI of normal children was 17.7±12.7, compared to 24.2±13.9 of obese children. Obese children had significantly higher mean fat mass, which was determined with BEI, 36.9%±7.1, than normal children, 19.8%±7.8. Obese boys and obese girls had mean fat mass of 38.5%±6.6 and 35.2%±7.6, receptively, compared to normal boys and girls, 18.1%±9.2, and 21.4%±6.4 ($p<0.05$), respectively.

As shown in Table 1, blood glucose was significantly higher in obese boys (91.8±11.7 mg/dl), compared to normal boys (88.3±8.4 mg/dl) ($p<0.05$); however, these values were within the normal range (70-110 mg/dl). In obese girls, the mean blood glucose was not significantly different than in normal girls. Blood GPT levels were significantly higher in both obese boys, 40.6%±13.7, and obese girls, 27.3%±16.4, compared to normal boys, 25.5%±11.5, and girls, 19.8%±11.8 ($p<0.05$). In obese boys, mean serum concentrations of TC (180±29 mg/dl) and TG (125.7±76.9 mg/dl) were significantly higher than TC (168±29 mg/dl) and TG (66.2±47 mg/dl) in normal boys ($p<0.05$). In obese girls, the mean serum concentrations of total cholesterol were higher than in normal girls. However, it was not significant. The concentration of TG was significantly higher in obese girls (101.7±64.4 mg/dl) than in normal girls (63.7±42.6 mg/dl ($p<0.05$). Regardless of sex, the concentration of HDL was similar in obese and normal children.

Table 1. Characteristics and biochemical analysis of normal and obese children

	Normal			Obese		
	Boys (88)	**Girls (82)**	**Total (170)**	**Boys (119)**	**Girls (57)**	**Total (176)**
Height (cm)	144.0± 7.9	143.7±16.6	144.8±13.0	148.3± 6.9	147.3± 7.7	147.8± 7.2
Weight (kg)	37.2± 8.0	37.6± 8.2	37.4± 8.0	55.7±10.5	48.5±10.9	53.4±11.2
Body fat mass (%)	18.1± 9.2	21.4± 6.4	19.8± 7.8	38.5± 6.6	35.2± 7.6	36.9± 7.1
BMI (%)[1]	17.9± 2.6	17.6± 2.6	17.7± 2.7	25.2± 3.5	22.2± 2.7	24.2± 3.9
B-GLU (mg/dl)[2]	88.3± 8.4	83.0±19.3	85.7±15.0	91.8±11.7*	81.9±20.2	88.6±15.6
GOT (IU/L)[3]	30.6± 9.1	26.5± 8.5	28.6± 9.0	32.7±31.0	27.0± 7.5	30. ±17.9
GPT (IU/L)[4]	25.5±10.5	19.8±11.8	22.7±17.0	40.6±13.7*	27.3±16.4*	36.2±37.5
TC (mg/dl)[5]	168.6±29.4	164.2±37.2	166.5±33.4	180.2±29.2*	176.5±44.2	179.0±34.8
HDL-C (mg/dl)[6]	49.4±22.0	56.7±26.1	52.9±24.3	48.0± 9.8	57.1±23.3	51.0±16.1
TG (mg/dl)[7]	66.2±46.9	60.9±37.6	63.7±42.6	125.7±76.9*	101.7±64.4*	117.9±73.8
LDL-C (mg/dl)[8]	115.0±39.3	130.4±68.7	122.7±54.0	140.3±49.7*	130.7±56.0	135.6±55.0
AI[9]	2.0± 0.7	1.6± 0.6	1.8± 0.7	2.8± 0.8	2.3± 1.2*	2.7± 0.9

Mean ages of Normal and obese children were 11.5±1.5yr. and 11.0±1.8yr.
( ): Number
All values are expressed as mean ± standard deviation
* : Significant difference, p <0.05, from t-test between normal and obese in boys or in girls.
1) Body Mass Index 2) Blood glucose 3) Glutamine oxaloacetic transaminase
4) Glutamic pyruvic transaminase 5) Total cholesterol 6) High density lipoprotein
7) Triglyceride 8) Low-density lipoprotein (LDL) cholesterol was calculated by (Cholesterol-TG)/5+ HDL
9) atherogenic index ((TC-HDL-C/HDL-C) (mg/mg)

Table 2. Concentration of serum antioxidant nutrients in normal and obese children

	Normal			Obese		
	Boys (88)	**Girls (82)**	**Total (170)**	**Boys (119)**	**Girls (57)**	**Total (176)**
Zn (ug/dl)	94.7 ±11.0	97.1 ±12.7	95.9 ±11.9	95.4 ±17.9	97.0 ±13.2	95.9±16.5
Retinol (mg/ml)	0.46± 0.12	0.48± 0.14	0.47± 0.14	0.44±10.14	0.49± 0.15	0.46± 0.15
Vitamin.E (mg/ml)	0.09± 0.02	0.07± 0.02	0.08± 0.02	0.10± 0.02	0.09± 0.01	0.08± 0.01
Vt.E/TC+TG (mg/mg)	0.36± 0.07	0.38± 0.08	0.37± 0.07	0.26± 0.05**	0.31± 0.04*	0.29± 0.04*

Mean ages of Normal and obese children were 11.5±1.5 yr. and 11.0±1.8 yr.
( ) : Number
Values are mean± standard deviation
* Significantly different between normal and obese (p<0.05, t-test) ** Significantly different between normal and obese (p<0.01, t-test)

Table 3. Correlation coefficients among various serum components and fat mass

	Fat mass	B-Glu[1)]	GOT[2)]	GPT[3)]	Zn	Retinol	Adjusted Vitamin E
Zn	-0.030	0.043	0.012	0.054	1.000	0.875**	0.959**
Retinol	-0.112*	0.077	0.095	0.046	-	-	0.904**
Adjusted Vtamin E	-0.156*	-0.013	-0.011	-0.059-		1.000	1.000

* : p<0.05 ** : p<0.01
1) Blood glucose 2) Glutamine oxaloacetic transaminase
3) Glutamic pyruvic transaminase

Table 2 shows the serum concentrations of vitamin E, retinol, and zinc. Before the adjustment of vitamin E for TG and TC, there were no statistical differences in serum concentrations of vitamin E between normal and obese children. However, after adjusting vitamin E for blood TG and TC, obese children showed significantly lower serum levels of vitamin E than normal children. Both obese boys and obese girls had significantly lower vitamin E concentrations than normal boys and girls ($p<0.05$). For serum retinol concentrations, obese children showed low retinol levels without reaching statistical significance. No significant differences between obese and normal children were found for serum zinc concentration. Zinc values in girls, regardless of obese status, seemed higher than those in boys, but not significant.

The correlation coefficients among blood components were shown in Table 3. Serum concentrations of retinol ($p<0.05$) and vitamin E ($p<0.05$) were negatively correlated with fat mass (%). Serum concentrations of retinol and vitamin E were significantly correlated with a value of $r=0.094$ ($p<0.01$). Correlation coefficient between vitamin E and Zn was 0.959 ($p<0.01$), while that between Zn and vitamin A was 0.875 ($p<0.01$).

DISCUSSION

The purpose of this study was to determine whether obese children have increased risk for chronic diseases such as diabetes, atherosclerosis, and obesity related liver diseases, as well as altered antioxidant nutrients status. This study also sought to determine whether those health risk factors are associated with antioxidant status. In this study, obese children showed higher blood glucose, GPT, total blood cholesterol and triglyceride than normal children ($p<0.05$, Table 1). Our results are consistent with other studies, which showed possible risks for chronic diseases in obese children.[3-9]

Among the antioxidant nutrients, vitamin E, retinol, and zinc, only serum vitamin E was significantly depleted in obese children ($p<0.05$, Table 2). Vitamin E is the most effective antioxidant vitamin.[38] Factors like dietary intake of vitamin E and blood lipid profiles are known to influence the blood vitamin E concentration.[38-40] This study showed that dietary intake of vitamin E in obese children was not related to serum vitamin E (data not shown). Blood vitamin E is also influenced by serum TG and TC concentrations.[39] Thus, serum vitamin E concentrations were divided by the sums of serum TC and TC. After the adjustment, significant differences in blood vitamin E between normal and obese children were found in this study ($p<0.05$, Table 2). These results suggest that serum vitamin E was not sufficient enough to play its antioxidant role in obese children, in spite of increased serum cholesterol and triglyceride, and thus obese children need to take more vitamin E containing foods.

Several studies have also reported lower antioxidant status in obese children.[17-20] Decsi *et al.*[18,20] reported lower serum concentrations of vitamin E and beta-carotene in a small number of obese children. Based on NHANES III data, Strauss also reported subnormal serum levels of beta-carotene and alpha-tocopherol in 726 obese children.[19] Kuno *et al.*'s study[17] reported that obese girls showed low antioxidant blood levels of beta-carotene and vitamin E. The exact mechanism by which obese children have low blood vitamin E status is not clearly understood at this time. It has been reported that increased oxidative stress in the obese is involved in the depletion of the body's antioxidant system.[38,39] Dandona *et al.*[41] reported that the obese had increased reactive oxygen species by leukocytes, and weight loss in the obese brought about the decrease in those free radical formations. Vincent *et al.*[42] reported that obesity predisposed the myocardium to oxidative stress and Okita *et al.*[43] reported that oxidative stress induced by the fatty liver of the obese Zucker rat was reduced by vitamin E administrations. Based on those studies and the antioxidant characteristics of vitamin E, we can't exclude the possibility that low serum vitamin E in obese children may be resulted due to increased oxidative stress in the obese.

Vitamin E is an important factor in protecting against LDL oxidation, which is one of the major causes of arteriosclerosis.[38] Kuno *et al.*'s study[17] reported that obese girls showed lower vitamin E levels and higher oxidation of LDL, compared to normal children. This study also found that serum vitamin E concentration was negatively correlated with fat mass (%) ($p<0.05$, Table 3). Therefore, if obese children are not careful about their weights, their serum vitamin E concentrations will become reduced, increasing their risk for atherosclerosis. To prevent this risk, consumption of foods rich in antioxidant vitamins, especially vitamin E, should be encouraged for obese children.

Retinol is a lipid soluble antioxidant, like vitamin E. However, in our study, serum retinol was not significantly different between normal and obese children (Table 2). Decsi *et al.*[20] reported that body fat content was correlated with plasma vitamin E but not with retinol, and suggested that factors influencing the availability of retinal and alpha-tocopherol in obese children might be different. However, some studies reported that obese participants had significantly lower vitamin A than normal weight participants.[44-46]

Lack of measurements for the serum concentration of carotenoids could be the limitation of this study for the following reasons; serum retinol concentration depends on the intakes of both retinol, animal source of vitamin A, and provitamin A, carotenoids in green and yellow vegetables and fruits.[45] A major source of vitamin A (retinol) in Korea is from vegetables and fruits,[47] and thus measuring serum carotenoids levels will be more accurately reflected vitamin A status in children.

One of the interesting nutrients in this study is zinc. Zinc is also a cofactor of superoxide dismutase (SOD), an antioxidant enzyme, and has an antioxidant role in several oxidative processes.[21] Several studies reported that zinc plays an important role in obesity metabolism, as the mediator of leptin, a hormone secreted by adipocytes.[25] Chen *et al.*[27] reported that zinc supplementation reduced the insulin secretion activity in obese mice.

Many adult obesity studies reported low concentrations of zinc in the obese.[21-24] Studies also reported that low levels of zinc in the obese were involved in the pathogenesis of chronic diseases such as diabetes and heart diseases.[26-27] Unlike zinc studies in the adult obese, studies of blood zinc levels in obese children have shown controversial results.[28-30] Yakinci *et al.*[28] reported that 41 obese children, aged 7-11, had significantly higher serum zinc compared to controls. Mrreiro *et al.*[29] found that the zinc levels in plasma and erythrocyte of 23 obese children were significantly lower than those in controls. Ditoro *et al.*[30] reported that the low blood level of zinc in 55 obese children was normalized after receiving a hypocaloric diet.

In this study, no differences of serum zinc between normal and obese Korean children and no correlations of zinc and other health indices were found. One possible explanation for the result of this study is that zinc deficiency is maybe related to the duration of obesity; therefore, it may not be evident in children. Chen *et al.*[24] suggested that lower blood plasma zinc level was not the short-term metabolic result.

Compared to the above other studies, this study recruited relatively large number of obese and normal children, and thus this can be a strength of this study, in which experimental errors of the study were minimized. However, to clarify zinc status in obese children, more studies about zinc and obesity are needed. Measuring SOD – zinc dependent antioxidant enzyme- activity, or measuring additional components of blood or hair zinc concentration, besides the measurement of serum zinc, will provide more accurate information for determining the role of zinc as antioxidant in children obesity.

In conclusion, obese Korean children showed higher risks of obesity related chronic diseases, and their serum vitamin E concentrations were significantly depleted. Therefore, careful monitoring of those health indices in obese children is needed.

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