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Plasma Micronutrients; Leptin and Lipid Profile in Obese Egyptian Adolescents: A Case Control Study

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ABSTRACT

Background*:* Childhood obesity is a global epidemic and rising trends in both developed and developing countries**.** Evidence has shown that obesity can be associated with substantial nutrient deficiencies. The prevalence of micronutrient deficiencies in obese individuals is higher compared with that in those with normal weight. ***Objective***: To assess micronutrients status, zinc (Zn); chromium (Cr), vitamin A (retinol) and vitamin E (α-tocopherol) as well as leptin and lipid profile in obese Egyptian adolescents. ***Design and subjects***: This was a case-control study with 80 obese adolescents and 80 healthy non-obese adolescents with matched age and gender as control. All subjects were subjected to anthropometric measurements including (weight, height, waist & hip circumference and percentile BMI) and blood pressure measurements. Plasma micronutrients including; Zn, Cr, retinol and α-tocopherol as well as lipid profile and plasma leptin were assessed. ***Results****:* The obese adolescents demonstrated significant differences in anthropometric indices Compared to control group. Plasma Zn and retinol were significantly lower in obese adolescents. Meanwhile, plasma leptin, total cholesterol (TC), triglycerides (TG) and High density lipoprotein (HDL-C) were significantly higher in obese adolescents. On the contrary, no significant differences were observed in plasma chromium, α- tocopherol and Low density lipoprotein (LDL-C) between obese and non-obese adolescents. ***Conclusion*****:** Obese children may be at a risk of developing imbalance (mainly deficiency) of some micronutrients, which may be playing an important role in the pathogenesis of obesity and related metabolic risk factors.

Keywords: Childhood obesity, Micronutrients, Leptin, Lipid profile

Introduction

Childhood obesity has become a global epidemic with important consequences for the health of future generations (Kelishadi *et al.,* 2012). During the last three decades, there has been a considerable increase in the prevalence of obesity in children and adolescents (4–18 year-old) worldwide (Kelishadi *et al.,* 2012; Gupta *et al.,* 2012; WHO, 2014). However, there is a wide variability in the prevalence of obesity and overweight among countries, ranging from 5% to 30% in 11-year-olds, and from 4 and 8% in both 13 and 15 year old girls and boys respectively to more than 30% in both ages. This is alarming because metabolic changes and the consequences of obesity, formerly observed only in the adults are now observed in younger individuals (Styne, 2001).

In Egypt, childhood obesity has been on the rise over the past 10 years. A study conducted by Salazar-Martinez *et al*. (2006) , showed that 12.1% of Egyptian adolescents (7% boys and 18% girls) were overweight and 6.2% (6% boys and 8% girls) were obese. Among Egyptian school children aged 8–12 years, the prevalence of overweight and obesity was 12.3 and 15.1%, respectively, in private schools and 13.4 and 6.7%, respectively, in public schools (Shaalan *et al.,* 2002).

Some studies suggested that micronutrient deficiencies may contribute to fat deposition and chronic inflammation (Garcia *et al.,* 2009, 2012) . A higher risk of low concentrations of iron, zinc, vitamin A, vitamin E and vitamin C have been observed in obese children and adolescents compared to children and adolescents with normal weight (Aeberli *et al.,* 2009 ; De Souza *et al.,* 2007). The deficiency of these micronutrients may increase the risk of developing obesity.

Leptin, a 16 kDa neurohormone predominantly synthesized and released into blood by adipocytes and serves as a signal for the brain of the body's energy store. Leptin controls food intake through its receptors in the hypothalamus by inhibiting the release of NPY which has an augmentative effect on food intake. By

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reducing food intake and increasing thermogenesis, leptin is a key hormone in the regulation of body weight and nutrition (Thaler and Schwartz, 2010).

Obesity is associated with several deleterious changes in lipid metabolism, including high serum concentrations of total cholesterol, LDL, VLDL and TG, and reduction in serum HDL concentration (Palou and Bonet, 2000). It has been showed that in all age groups, HDL levels were significantly lower in patients who had a high BMI (Rosenson, 2007).

In this study, we aimed to estimate plasma micronutrients status, zinc (Zn); chromium (Cr), vitamin A (retinol) and vitamin E (α-tocopherol) as well as leptin and lipid profile in obese Egyptian adolescents in comparison with normal weight controls.

Subjects and methods

Study Patients

This case-control study that was conducted on 80 obese adolescents (girls and boys and 80 age and sex matched normal-weight adolescents (control group). The obese adolescents were recruited from obesity unite of the outpatients clinic of National Nutrition Institute, while the control group was taken from private clinic. All subjects were given detailed information about the aims of the study, and written consent forms were obtained.

Eligibility criteria

Obese adolescents were being obese, as defined by WHO percentile body mass index "BMI"/age for males and females cut points (WHO, 1995). The age must have been between 12 and 18 years.

Exclusion criteria

Adolescents with extreme obesity (BMI z score > 4), syndromal obesity, endocrine disorders, any physical disability, history of chronic diseases and/or chronic medications use, use of minerals and/ or vitamins or supplementation or children under special diets were not included in the study.

Anthropometric measurements and blood pressure

Anthropometric measurements including body weight measured to the nearest 0.1 kg with a balance scale and height measured to the nearest 0.1 cm. The waist circumference was measured at its smallest point between the iliac crest and rib cage, and the hip circumference was measured at its largest width over the greater trochanters. All the measurements were repeated twice.

For adolescents from 12-18 years old the weight the percentile body mass index "BMI"/age for males and females were used (WHO, 1995). The following categories of weight status were determined: Normal weight: 5^{th} - $<85^{th}$ percentile; Overweight: 85th - <95th percentile and Obese: $\geq 95^{th}$ percentile. Height status for adolescents was assessed using NCHS reference standard and Z- scores. The following categories of height status were determined: Stunting <-2SD; Normal– 2 to + 2SD and Tall: > + 2SD.

Blood pressure was measured in duplicate and high blood pressure was defined with systolic or diastolic blood pressure higher than the 95^{th} percentile (NHBPEP, 2004) .

Laboratory analysis

Blood samples of 5 ml were collected in heparinized tubes from all subjects after overnight fasting Plasma was separated by centrifugation and kept frozen for further analysis. The following biochemical tests were done for all subjects:

Determination of minerals:

Plasma Zn and Cr were determined by atomic spectrophotometry as described by Wang and Taylor, (1980) and Thomerson and Price, (1971) respectively.

Determination of vitamins:

Vitamins A and E were simultaneously measured in plasma using the method of Bieri *et al.* (1979).

Measurments of Leptin:

Using the Leptin (sandwich) Enzyme immunoassay kit. This assay is intended for *in vitro* diagnostic use only. It is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle using DRG ELISA kit (Considine *et al.,* 1996) .

Determination of Lipid profile:

TG, TC and HDL-C were determined in plasma according to Fossati and Prencipe, (1982); Allain *et al.,* 1974; and Finley *et al.,* 1978)) respectively. LDL- C was calculated according to the equation of Friedewald *et al*., (1972).

Statistical analysis:

Data were analyzed by SPSS statistical package version 17. Excel computer program was used to tabulate the results. Independent *t*-test was used to declare the significant difference between each two groups at $P<0.05$ (Stanford and Charles, 2003) .Results were expressed as means ± SD.

Results

This study included 80 obese adolescents (41 girls and 39 boys), age ranged from 12 to 18 years with mean age of 14.5±1.8 years, and 80 non-obese control adolescents (39 girls and 41 boys), mean age 14.1 ± 1.9 years, whose clinical characteristics are listed in Table 1. The obesity and control groups showed no significant differences in terms of gender and age ($P>0.05$).

Compared to the controls, the obese adolescents demonstrated significant differences in anthropometric indices including body weight, height, waist circumference, hip circumference and percentile BMI ($p < 0.05$; Table 1). However, the difference in height between girls and their corresponding control was non-significant.

Table 1: Clinical parameters of obese adolescents categorized by weight status and gender (Mean±S.D).

Parameters	Males		Female		Total	
	Normal (41)	Obese (39)	Normal (39)	Obese (41)	Normal (80)	Obese (80)
Age (year)	13.9± 1.91	14.3± 2.12	14.2± 2.30	14.4± 2.20	14.1±. 2.21	14.5± 1.80
Weight	58.4± 8.33	92.1± 11.04 [a]	55.5± 8.15	87.1± 10.20 [a]	56.6± 9.11	97.0± 10.33
Height	160.6± 11.19	165.3± 9.94[a,b]	160.1± 6.16	158.7± 8.67	161.0± 10.5	166.3± 9.84 [a]
Waist circumference (cm)	76.0± 10.94	99.6± 12.70[a]	69.6± 9.70	99.7± 12.60 [a]	85.3± 10.42	100.31± 12.31
Hip circumference (cm)	90.7± 13.6	112.2± 18.4 [a]	90.9± 13.7	115.5± 11.3 [a]	91.2± 14.00	117.0± 15.41 [a]
Percentile BMI	22.7± 3.59	33.5± 4.14 [a]	21.6± 2.81	34.8± 4.8 [a]	22.1± 3.31	34.6± 4.50 [a]
Systolic blood pressure	107.3± 15.71	118.7± 16.60 [a]	106.7± 15.81	115.9± 14.10 [a]	107.0±16.00	119.01± 14.82 [a]
Diastolic blood pressure	69.9± 11.4	77.2± 13.9 [a]	68.3± 11.1	77.1± 14.6 [a]	66.3± 10.86	78.0± 14.21 [a]

a): Significant differences between weight status. (b): Significant differences between sexes . P < 0.05 BMI: Body mass index

The effect of gender on anthropometric measurements (table, 1) showed that with the exception the mean values of height, all other measurements exhibited non-significant differences between both sexes

Systolic and diastolic blood pressures were significantly higher in obese adolescents compared with their corresponding controls ($P < 0.05$; table, 1). However, there were no significant differences in systolic and diastolic blood pressure between both sexes.

Plasma levels of Zn and retinol were significantly lower in obese adolescents (table, 2, $p < 0.05$). Moreover, plasma Zn of boys and retinol levels of girls were significantly lower than their controls. However, no significant differences were observed in serum levels of Cr and α-tocopherol between all cases of obese adolescents and their corresponding controls ($p > 0.05$).

As shown in table (2), plasma levels of leptin TC and TG was significantly higher ($p < 0.05$) in obese adolescents (total, boys and girls) as compared to non-obese ones. On the contrary, mean HDL-C values were significantly higher in all cases of obese adolescents as compared to their corresponding controls. No significant differences were observed in TC, TG and HDL-C between boys and girls. Moreover, plasma LDL-C levels of all cases (total, boys, girls) exhibited insignificant differences as compared to their corresponding controls.

Table 2: Biochemical parameters of obese adolescents categorized by weight status and gender (Mean±S.D).

Parameters	Males		Female		Total	
	Normal (41)	Obese (39)	Normal (39)	Obese (41)	Normal (80)	Obese (80)
Zinc (mg/dl)	14.3± 3.0	13.0± 2.3[a]	12.5± 2.21	12.37± 2.06	13.7± 2.5	12.8± 2.1[a]
Chromium (µg/dl)	0.25± 0.07	0.36 ± 0.1	0.3± 0.09	0.33± 0.11	0.29± 0.06	0.35± 0.11
Retinol (µg/dl)	49.2± 8.5	45.2± 9.3 [a]	58.1± 6.82	46.6± 8.04 [a]	53.0± 9.5	45.7± 17.5[a]
α-tocopherol (µg/dl)	1043± 169	1031± 124	1070± 178	1034± 193	1039± 148	1065± 162
Leptin (ng/dl)	2.93± 0.51	3.58± 0.35 [a, b]	3.05± 0.35	3.42± 0.58 [a]	3.12± 0.47	3.60± 0.53 [a]
TC (mg/dl)	153.02± 18.10	161.98± 21.9 [a]	158.41± 19.92	167.95± 21.4 [a]	155.24± 22.7	164.58± 20.4 [a]
TG (mg/dl)	102.81± 21.51	115.20± 20.62 [a]	96.10± 20.40	108.60± 21.7 [a]	99.23± 19.6	110.81± 20.3 [a]
HDL-C (mg/dl)	45.60± 8.5	47.57± 7.57	44.1± 8.7	49.0± 7.7 [a]	43.3± 7.4	48.1± 9.1 [a]
LDL-C (mg/dl)	86.4± 13.96	91.6± 14.9	91.97± 15.8	95.4± 15.4	91.5± 13.8	93.7± 14.9

(a): Significant differences between weight status. (b): Significant differences between sexes. P <0.05 TC: Total cholesterol. TG: Triglyceride. HDL-C: High density lipoprotein
LDL-C: Low density lipoprotein

Discussion

Zinc, as an important micronutrient, plays a key role in macronutrient metabolism (Song *et al.,* 2005) as well as appetite control. In addition, zinc is involved in synthesis, storage, release, and action of insulin (Simon and Taylor, 2001) and its deficiency is associated with insulin (Tallman and Taylor, 2003).

The results of our study showed that serum Zn levels were significantly reduced in obese adolescents. Similar results were reported by Azab *et al.* (2014) in obese Egyptian children and by Yerlikaya *et al.* (2013) in obese women. On contrast, Bougle *et al.* (2009) found no significant differences in Zn levels between obese and non-obese children.

Zn status, particularly circulating level, is known to be altered in conditions such as obesity (Klevay, 2010) . Obese people experience chronic inflammation resembling that found in infections (Prasad *et al.,* 2007) . Studies showed that Zn deficiency increases the concentration of inflammatory cytokines (Dambal *et al.,* 2011) . Zn metabolism is linked to obesity: in obese patients it is inversely related to leptin and protects against hyperglycemia and insulin resistance (Mantzoros *et al.,* 1998).

Zinc, in particular, takes part in the metabolism of hormones involved in the pathophysiology of obesity. It has been observed that zinc concentration is directly associated with serum leptin concentration, an adipokine associated with satiety (Mracek *et al.,* 2009) . This association could be explained by the effect of zinc-α 2-glycoprotein (ZAG) on leptin concentrations. ZAG is an adipokine involved in lipolysis in the adipocyte that is down-regulated in obesity. In obese individuals, low ZAG gene expression is associated with low serum adiponectin and high plasma leptin levels, and may play an important role in the

pathogenesis of obesity (Mracek *et al.,* 2009). Chen and Lin, (2000) suggested in their study that leptin resistance that occurred in obesity might have resulted from zinc deficiency. On the contrary, no differences were observed in zinc concentrations between obese and non-obese Turkish children (Tascilar *et al.,* 2010).

The significance of chromium as a trace nutrient is well documented and its function in the control of glucose and lipid metabolism has been claimed (Balk *et al.,* 2007). Our results indicated no association between serum chromium levels and obesity.

The mean value of retinol was significantly lower in obese children than in non-obese ones. This was in agreement with the results of Ismail *et al.* (2014) who found that vitamin A (retinol) was significantly lower in obese children ($P < 0.000$). Moreover, the results of Botella-Carretero *et al.* (2010) showed that serum retinol insufficiency was statistically significant between cases and controls, also the results of Suano de Souza *et al.* (2008), revealed that the risk for obesity was related to a trend to a higher percentage of retinol inadequacy in obese children. In contrast, Sarni *et al.* (2005) found an insignificant difference in serum retinol insufficiency between obese and non-obese Brazilian children. This could be attributed to the fact that the Brazilian diet is different from the Egyptian diet and to the difference in sample size.

Leptin is a hormone made by adipocytes & mainly acts centrally to control body weight Schlienger, (2004) . It conveys information to the brain about the size of energy stores & stimulates the hypothalamic centers responsible for regulation of energy intake & expenditure (Ahima and Flier, 2000) . Plasma leptin levels in humans are strongly correlated with the Body Mass Index (BMI) and total fat mass and are mostly elevated in obese subjects (Kohrt *et al.,* 1996).

The increased levels of serum leptin concentration observed in the present study was in accordance with those of Azab *et al.,* (2014) in obese children and Turki *et al.,* (2009) in obese adult.

Leptin is expressed predominantly by adipocyte, which fits the idea that body weight is sensed as a total mass of fat in the body (Wauters *et al.,* 1998) . Serum leptin concentration is increased in obese. subjects and is closely related to fat mass and BMI and declines with weight loss (Considine *et al.,* (1996).

Leptin plays a central role in the long-term maintenance of weight homeostasis by acting on the hypothalamus to decrease food intake and increase energy expenditure Flier *et al.,* (2005). Minocci *et al.* (2000) concluded that fat distribution contributes to the variability in serum leptin in obese patients. In particular, subcutaneous abdominal fat is a determinant of leptin concentration.

The inability of such elevated leptin levels in most obese individuals to alter the obese state of subjects may be related to "leptin resistance " (Emanuelli *et al.,* 2001), an inability of leptin to enter the cerebral spinal fluid to reach the hypothalamus regions that regulate appetite, or it may simply reflect the large amount of fat tissue in the body (Ahmad *et al.,* 2001).

In our study, significantly increased serum levels of TG & TC and significantly decreased levels of HDL-C than healthy controls were in agreement with other investigators (Azab *et al.,* 2014; Lima *et al.,* 2009). In contrast, Woo *et al.* (2004) studied a cohort of obese subjects of Chinese ethnicity that did not show elevated cholesterol levels compared to a control group.

Hypertriglyceridemia is often associated with reduced levels of HDL suggesting a possible metabolic interaction between these two lipid fractions (Rosenson, 2007). The key to this relation may be that the increase in fat deposition in obese individuals is associated with insulin resistance (Ginsberg and Stalenhoef, 2003) , which will lead to increase synthesis of TG-rich lipoproteins in the liver. The increase of TG in lipid particles changes their metabolism. TG-rich HDL particles are hydrolyzed more rapidly causing HDL level to fall (Carr and Brunzell, 2004).

Some investigators demonstrated that the decreased levels of Zn in serum as well as the significant elevation in lipid parameters in obese children indicate the possible effect of Zn level on serum lipid profile which may be due to the role of Zn as an antioxidant. Thus, the decrease in Zn level in obese children may lead to increased lipid peroxidation and leading to increased levels of TC and TG (-Azab *et al.,* 2014; Bouglé *et al.,* 2009). These results are concordant with the report of Gunasekara *et al.* (2011), who found that treatment with Zn reduced TC, TG, and LDL-C plasma levels and increased HDL-C levels

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