Leptin and Insulin Resistance in Obese Children

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ABSTRACT

Background: Insulin resistance (IR) is the primary metabolic disorder associated with obesity. Obesity is a growing worldwide health problem affecting both adults and children.

Objectives: To determine the association between leptin and IR, and to identify the ratio of fasting glucose/leptin (G/L) and insulin/leptin (I/L) as a new simple method for the detection of IR in obese children.

Methods: This study was done in the National Diabetic Center/ AL-Mustansiriya University during the period from May 2013 until the end of October 2013. Fasting blood glucose (FBG), serum insulin, leptin, and lipid profile were measured in 52 obese children (24 children with IR and 28 without IR); their age range was (5-15) years, they were compared with 38 healthy children as a control group.

Results: Means of FBG, insulin, leptin, total cholesterol (TC), triacylglycerol (TAG), low density lipoprotein cholesterol (LDL-C), and non high density lipoprotein cholesterol (non HDL-C) were significantly increased in obese children with IR as compared in children without IR, ($P<0.05$), while there was a significant decrease in serum level of high density lipoprotein cholesterol (HDL-C) in obese children with IR when compared with obese children without IR, ($P=0.001$). There was a decrease in the ratio of G/L and an increase in the ratio of I/L in obese children with IR, but it was not significant. A significant positive correlation was found between serum leptin verse body mass index (BMI), FBG, insulin, homeostasis model assessment for insulin resistance (HOMA-IR), I/L ratio, TC, TAG, LDL-C, and non HDL-C, while a significant negative correlation was found between serum leptin and HDL-C in obese children with IR.

Conclusions: The present results showed that serum leptin is correlated with BMI, FBG, insulin, HOMA-IR, I/L ratio, TC, TAG, LDL-C, and non HDL-C in obese children with IR. The G/L ratio can be used in addition to me /L ratio, and HOMA to accurately assess IR in obese children.

Key Words: Obesity, insulin resistance, Leptin.

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Insulin resistance (IR) is a state in which normal concentrations of insulin produce a subnormal biologic response [1]. It has been associated with the rising prevalence of many metabolic complications, such as hyperlipidemia, hyperglycemia and high blood pressure [2].

Childhood obesity, which is increasing worldwide, is well known for its association with IR [3]. Several modifications of the body composition occur, such as the increase in intramuscular fat content and altered expression of genes important in muscle growth and metabolism, leading to IR [4].

Puberty is a critical period for the development of metabolic syndrome (MS), because of the physiological resistance to insulin, which is accompanied by an increase in body fat percentage, changes in blood pressure and serum lipids, accentuated by a sedentary lifestyle and overeating [5]. Complex metabolic changes, acting in a mutually linked manner, are the basic cornerstones for the development of MS that are characterized by IR, endothelial dysfunction and dyslipidemia [6].

The impairment of the homeostatic relation between plasma insulin and glucose concentrations is a symptom of insufficient insulin efficiency that can be evaluated using the so-called homeostatic model for IR assessment (HOMA-IR), which is derived from fasting plasma glucose and fasting plasma insulin concentrations and correlates reasonably with the euglycemic clamp technique [7].

Insufficient leptin signaling in the hypothalamus, which is caused by either decreased availability of leptin for transport to the hypothalamus (in the case of leptinopenia), or restricted leptin entry across the blood brain barrier (imposed by hyperleptinemia in obese subjects), is primarily responsible for inducing hyperglycemia and hyperinsulinemia [8].

Leptin is a protein containing 166 amino acids and is the product of human obese (Ob) gene. Leptin is produced mainly by adipose tissue. It is also synthesized in small amounts in other human tissues such as the stomach, heart, mammary epithelium and placenta. Leptin acts through the leptin receptor (LEPR or OBR) [9]. Effects of leptin on various systems have been reported, including reproduction, immune system, hematopoesis, angiogenesis, bone formation and wound healing. Serum leptin levels are elevated in obese children; besides, leptin levels decrease during the weight loss period [10]. Leptin action in the brain potently suppresses hepatic glucose production while increasing tissue glucose uptake despite persistent, severe insulin deficiency [11].

In this context, the aim of study was to evaluate IR by determination of HOMA-IR, glucose to leptin (G/L) ratio and insulin to leptin (I/L) ratio values also to find the association with each of leptin and lipids in obese children.

Methods. This study was done in the National Diabetic Center/AL-Mustansiriya University during the period from May 2013 until the end of October 2013. About 5 milliliters of venous blood were obtained from 52 obese children (24 children with IR and 28 without IR); their age range was (5-15) years, they were compared with 38 healthy children as control group.

Exclusion Criteria: All information was obtained directly by medical history in a private interview. All subjects who were using any medications and subjects with renal diseases, liver diseases, malignant disorders, diabetes mellitus, and diseases of the pituitary gland or thyroid gland, were not considered to be in this study.

Measurements:

1- Anthropometric Measurements: Body mass index was calculated by dividing subjects weight (Kg) by their height

(m^2). BMI calculated as: BMI= mass (kg)/(height m^2) [12].

2- Biochemical Assessment:

Determination of Fasting Blood Glucose (FBG): Glucose was determined, by using the enzymatic colorimetric method (GOD-POD) [13].

Determination of Serum Total Cholesterol (TC): Serum TC was measured by cholesterol kit, using an enzymatic method [14].

Determination of Serum Triacylglycerol (TAG): Serum TAG was measured by TAG kit, using an enzymatic method [15].

Determination of Serum High Density Lipoprotein Cholesterol (HDL-C): Serum HDL-C was measured by HDL-C kit, using an enzymatic method [16].

Estimation of Serum Low Density Lipoprotein Cholesterol (LDL-C): Serum LDL-C was calculated indirectly by using the Friedewald's equation [17]. LDL-C = TC – [HDL-C + TAG/5]. This equation is only accurate when TAG levels are below 400 mg/dl.

Estimation of Serum Non High Density Lipoprotein Cholesterol (Non HDL-C): Serum non HDL-C was calculated directly from the difference between serum total and high density lipoprotein cholesterol [18].Serum non HDL-C= (serum TC – serum.HDL-C).

3-*Hormonal Assessment:*

Determination of Serum Insulin: Serum insulin concentrations were measured by the DRG insulin ELISA kit [19].

Estimation of IR: Insulin resistance was calculated by using the homeostasis model assessment for IR (HOMA-IR) [20].

(HOMA-IR) = {[fasting insulin (µU/ml)] × [fasting glucose (mg/dl)]}/405. The constant 405 should be replaced by 22.5 if glucose is expressed in S.I. units.

Determination of Serum Leptin: Serum leptin concentrations were measured by the DRG leptin ELISA kit [21].

Statistical Analysis:

All the statistical work and registration of obtained data were carried out by using Microsoft office excel 2010 work sheet. Data were expressed as means (±standard deviation [SD]). Differences considered of statistical significance according to the t-test at P<0.05.

Results. Characteristic of obese children and control group were summarized in table 1. Body mass index (BMI), FBG, insulin, HOMA-IR, leptin, TC, TAG, LDL-C, and non HDL-C were significantly increased in obese children when compared with the control group, while there was a significant decrease in serum HDL-C when compared with the control group. There was no significant difference in age, G/L ratio, and I/L ratio between obese and control group.

Table 2 shows comparison between obese boys and girls. There was a significant increase in BMI, FBG, insulin, leptin, TC, TAG, and HDL-C in obese girls' children when compared with obese boys' children.

Table 3 shows comparison between obese children with and without IR. There was a significant increase in FBG, insulin, leptin, TC, TAG, LDL-C, and non HDL-C in obese children with IR as compared with obese children without IR, (P<0.05), while there was a significant decrease in serum HDL-C in obese children with IR as compared to obese children without IR, (P=0.001). Table 4 shows a significant positive correlation between serum leptin verse BMI, FBG, insulin, HOMA-IR, I/L ratio, TC, TAG, LDL-C, and non HDL-C in obese children with IR, while a significant negative correlation was found between serum leptin verses HDL-C in these populations.

Discussion. Obese children and adolescents are more likely to have serious health conditions, such as cardiovascular, metabolic and psychosocial illnesses [22]. Regarding the adverse effects of obesity in particular the visceral obesity on glucose metabolism many probable mechanisms have been suggested which include, excessive lipid supply by a mechanism currently referred to as lipotoxicity. When free fatty acids are elevated for a prolonged period, they have a direct effect on insulin action in skeletal muscle tissue and liver, reducing the normal responses to insulin to promote glucose uptake and to suppress hepatic glucose output, respectively [23].

Obesity leads to IR and increased circulating insulin concentrations over time, thereby decreasing insulin sensitivity and impairing pancreatic β-cell function [24]. The importance of HOMA-IR index as an adequate tool for determination of IR in obese children was further supported by Maknie et al., [25]. However, the glucose/insulin (G/I) ratio does not appropriately reflect the physiology underlying the determinants of insulin sensitivity [26]. For example, given the same level of relative fasting hyperinsulinemia in a diabetic and a non diabetic IR subject, 1/(fasting insulin) remains unchanged. However, under these same conditions, the G/I ratio paradoxically and erroneously increases in the diabetic subject. Therefore, the fasting G/I ratio is a conceptually flawed index of insulin sensitivity. The fasting G/I ratio is a theoretically imperfect index of insulin sensitivity [27].

Girls presented the highest insulin levels and HOMA-IR values, along with lower glucose concentration. The

Table 1: Characteristics of obese children and control group.

Parameters	Obese Children (n=52)	Control (n=38)	P value
Gender (M/F)	(25/27)	(18/20)	-
Age (Years)	11.30±4.11	11.02±5.41	0.690 NS
BMI (Kg/m^2)	31.10±6.02	25.10±5.10	0.01
FBG (mg/dl)	96.20±6.41	80.31±8.21	0.001
Insulin (µU/ml)	17.05±4.16	6.80±2.11	0.0001
HOMA-IR	4.06±2.32	1.35±0.13	0.0001
Leptin (ng/ml)	27.40±10.20	18.50±9.60	0.0001
G/L ratio	3.51±1.33	4.34±2.42	0.50 NS
I/L ratio	0.62±0.41	0.38±0.22	0.420 NS
TC (mg/dl)	180.91±12.31	146.03±5.50	0.0001
TAG (mg/dl)	141.29±9.50	87.11±5.14	0.003
HDL-C (mg/dl)	47.54±7.05	57.11±6.71	0.001
LDL-C (mg/dl)	105.11±6.39	71.50±5.05	0.0001
Non HDL-C (mg/dl)	133.37±9.44	88.92±8.52	0.001

NS: not significant.

Table 2: Comparison between obese boys and girls.

Parameters	Obese Children		P Value
	Girls (n=30)	Boys (n=22)	
Age (Years)	11.26±3.15	11.34±3.06	0.120 NS
BMI (Kg/m^2)	33.70±4.19	28.50±4.16	0.045
FBG (mg/dl)	98.50± 4.12	93.90±6.30	0.045
Insulin (µU/ml)	18.30±3.41	15.80±0.24	0.045
HOMA-IR	4.45±0.90	3.66±0.16	0.131 NS
Leptin (ng/ml)	33.29±3.20	21.51±2.88	0.01
G/L ratio	2.96±1.78	4.37±2.06	0.261 NS
I/L ratio	0.55±0.33	0.73±0.12	0.310 NS
TC (mg/dl)	184.68± 9.13	177.14±12.0	0.045
TAG (mg/dl)	150.36±0.61	132.21 ±0.80	0.01
HDL-C (mg/dl)	50.18±5.66	44.71± 4.28	0.045
LDL-C (mg/dl)	104.43±6.60	106.11± 9.14	0.451 NS
Non HDL-C (mg/dl)	134.50±8.02	132.43±6.05	0.110 NS

NS: not significant.

Table 3:. Comparison between obese children with and without IR.

Parameters	Obese Children		P Value
	With IR (n=24)	Without IR (n=28)	
Gender (M/F)	)10/14(	(16/12)	-
BMI (Kg/m^2)	32.40±2.10	29.80±1.90	0.70 NS
FBG (mg/dl)	98.80±3.80	93.60±1.20	0.045
Insulin (µU/ml)	20.40±0.50	13.05±3.10	0.01
HOMA-IR	5.0±0.13	3.12±0.09	0.340 NS
Leptin (ng/ml)	32.30±4.40	22.50±2.80	0.02
L ratio/ G	3.06±4.80	4.16±4.20	0.830 NS
L ratio/I	0.63±1.10	0.60±0.90	0.160 NS
TC (mg/dl)	186.02±7.20	175.80±6.90	0.045
TAG (mg/dl)	150.07±0.50	132.51±4.60	0.001
HDL-C (mg/dl)	43.31±8.10	51.59±3.0	0.001
LDL-C (mg/dl)	112.70±0.60	97.71±2.50	0.01
Non HDL-C (mg/dl)	142.71±0.41	124.21±7.25	0.01

NS: not significant.

Table 4. Correlations between leptin and different parameters in obese children with IR.

Parameters	Leptin	
	R	P
Age (Years)	0.102	0.636 NS
BMI (Kg/m^2)	0.728	0.001**
FBG (mg/dl)	0.913	0.001**
Insulin (µU/ml)	0.619	0.045*
HOMA-IR	0.608	0.04*
G/L ratio	0.223	0.356 NS
I/L ratio	0.969	0.001**
TC (mg/dl)	0.426	0.045*
TAG (mg/dl)	0.401	0.045*
HDL-C (mg/dl)	-0.613	0.045*
LDL-C (mg/dl)	0.502	0.02*
Non HDL-C (mg/dl)	0.436	0.045*

NS: not significant.

hyperinsulinemia might be caused by the impairment of leptin singling in pancreatic β-cell and contributes to obesity and IR [28]. Leptin has provided an important relationship between energy homeostasis and regulation of reproduction

Leptin is produced by adipose cells and plays an important role in the regulation of body weight and metabolism [29, 30].

In present study, as expected, children with excess weight expressed a significantly increased level of leptin, which is in agreement with the study of Muc et al., [31]. It has been shown that leptin levels are increasing with puberty (girls). This pattern could be a consequence of the fact that, at equal ages, girls can enter puberty up to two years earlier than boys [32]; therefore, girls would have reached higher pubertal stages early than boys. In this study, girls had significantly higher leptin levels, Garcia-Mayor et al. showed, in normal weight children aged 5-15 years, that leptin levels in boys were always lower than in girls,

although they increased with age [33]. The lower leptin levels in boys may be partly explained by the suppressive effects of androgen.

A new ratio of fasting G/L was proposed in this study to be used as an index for predicting IR in obese children when fasting glucose levels are abnormal, which is in agreement with the study of Baban et al.[34]. Also the ratio of I/L was used in this study to predict IR in obese children. In accordance with previous study [35], on adolescents and adults, the IR reported in this study is associated with the primary alterations in the lipid profile; hyperinsulinism, increases the TAG synthesis also this study demonstrated that HDL-C was significantly lowered in obese as compared to non obese children.

Leptin had a positive correlation with BMI. Also this hormone was considerably associated with the other MS parameters such as FBG, insulin, HOMA-IR, I/L ratio, TC, TAG, LDL-C, and non HDL-C.

This study found that girls had higher serum non HDL-C levels than boys, which is in agreement with the study of Fang et al. [36]. In conclusion, this study showed that serum leptin levels was increased in obese children and had a relationship with increased IR and that it led to other diseases in the future such as diabetes mellitus and cardiovascular diseases; gender was influencing factors. This study also found that obesity was a factor of high non HDL-C levels in children and adolescents.

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