

Adipocytokine Profile and Insulin Resistance in Childhood Obesity

Iuliana GHERLAN^a; Suzana VLADOIU^b; Florin ALEXIU^c; Mihaela GIURCANEANU^b;
Sabina OROS^b; Andreea BREHAR^a; Camelia PROCOPIUC^a;
Constantin DUMITRACHE^d

^a“C.I.Parhon” National Institute of Endocrinology - Pediatric Endocrinology Dpt.,
Bucharest, Romania

^b“C.I.Parhon” National Institute of Endocrinology - Scientific Research Dpt.,
Bucharest, Romania

^c“C.I.Parhon” National Institute of Endocrinology - Nuclear Medicine Dpt.,
Bucharest, Romania

^d“C.I.Parhon” National Institute of Endocrinology - Clinical Dpt. Bucharest, Romania

ABSTRACT

Background: Adipose tissue is a veritable “endocrine organ” due to its adipocytokines secretion implied in insulin sensitivity modulation and cardiovascular complications.

Objective: To identify the adipocytokines’ plasmatic profile (adiponectin, leptin, resistin, IL-6, TNF α) in obese children and adolescents and to assess their relationship with “classic” clinical/paraclinical markers of metabolic syndrome and insulin resistance.

Material and Methods: A case-control study comparing a study group of 38 obese children and adolescents (age 13.5 \pm 2.3 years) to a normal weight age matched control group of 24 children.

We measured body mass index (BMI) and waist circumference (WC), systolic and diastolic blood pressure (BP). The classical metabolic parameters (fasting glycemia, total cholesterol and its fractions, serum triglycerides) were measured in both groups. Insulin sensitivity was evaluated using fasting insulinemia, HOMA-index and insulin-resistance summary score (IRS). Adiponectin, leptin, resistin, IL-6 and TNF α were measured using ELISA method.

Outcomes: Serum levels of leptin, resistin and IL-6 were significantly higher (42.42 \pm 22.58 ng/ml versus 14.4 \pm 14.49 ng/ml, $p < 0.001$; 9.69 \pm 3.47 ng/ml versus 7.92 \pm 2.13ng/ml, $p = 0.029$ and 2.66 \pm 2.87 pg/ml versus 0.89 \pm 1.16 pg/ml, $p = 0.006$ respectively), while adiponectin levels were significantly lower (9.05 \pm 4.61 μ g/ml versus 15.93 \pm 9.24 μ g/ml, $p < 0.001$) in the obese group compared to control group. TNF α was not statistical different between groups.

In multivariate regression analysis adiponectin was negatively and significantly correlated with WC ($r = - 0.463$, $p = 0.003$); leptin was positively and significantly related to WC, diastolic BP, fasting insulinemia and resistin ($r = 0.775$, $p < 0.001$); resistin was positively related to leptin and IL-6 ($r = 0.499$, $p < 0.001$), IL-6 was positively and significantly related to diastolic blood pressure ($r = 0.333$, $p = 0.008$).

Address for correspondence:

Iuliana Gherlan, PhD, “C.I.Parhon” National Institut of Endocrinology – Pediatric Endocrinology Dpt., Blvd. Aviatorilor No. 34-36, Sector 1, Bucharest, 011863; Phone number +40722204400; Fax +4021-3170607
E-mail: iuliana_gherlan@yahoo.com

Article received on the 20th August 2012. Article accepted on the 15th September 2012.

Conclusions: Serum levels of adiponectin, leptin, resistin and IL-6 are significantly different in obese children compared to normal weight controls; leptin was the only adipokine correlated with insulin resistance in children. There are significant correlations between plasmatic levels of leptin, resistin and IL-6.

Simple plasmatic determination of TNF α is not a marker of the degree of obesity or its metabolic complications in pediatric population.

Keywords: adipokine, cytokine, obesity, children

INTRODUCTION

Pediatric obesity has become an epidemic health issue in industrialized regions. Data from the NHANES (National Health and Nutrition Examination Survey) survey (2007–2008) indicate that approximately 9.5% of infants and toddlers were at or above the 95th percentile of the weight-for-recumbent-length growth charts, 11.9% of children and adolescents aged 2 to 19 years were severely obese (at or above the 97th percentile of the BMI-for-age growth charts), 16.9% were obese and 31.7% were overweight (1); the European Association for the Study of Obesity (EASO) has recently shown that overweight prevalence in European pediatric population (age 7-17 years) was 16-22%, while 4-6% of children and adolescents were already obese (2). The proportion of overweight and obese children and adolescents near doubled in 2010 compared to that reported in 1990-2003. It is estimated that in European Union almost 1.3 million children become overweight annually and that obesity incidence in the pediatric population is 300 000 new cases/year (3).

Obesity is associated to a metabolic risk which evolves to a cardiovascular risk. Obesity complications, as type 2 diabetes, dyslipidemia, arterial hypertension - formerly recognized in adult obese population - have recently been described in obese children and adolescents, with continuous growing prevalence, paralleling those of overweight prevalence (4-7).

Insulin resistance has a defined role in metabolic complications of obesity. There are several molecular mechanisms concurring to its appearance – recent years papers have demonstrated that various adipokines and cytokine derived from adipocytes per se or from others cellular elements of stromal or vascular origins in adipose tissue are responsible for insulin resistance and metabolic alterations in obese persons.

Adiponectin is the main adipokine secreted by adipocytes and the only adipokine down regulated in obesity. It has insulin-sensitizing effects through multiple mechanisms (inhibits hepatic gluconeogenesis (8), increases glucose uptake in adipocytes (9) and muscle cells (10) and protective cardiovascular effects (diminishes adhesion molecules expression, smooth muscle cells proliferation, suppress macrophage transformation in foam cells, has direct antithrombotic effects (11-14), stimulates nitric oxide production in small vessels (15,16).

Plasmatic levels of adiponectin were negatively correlated with BMI, adipose tissue proportion and fasting insulinemia in different adult populations (17-24) and also in pediatric populations: Pima Indians children (25), Japanese children (26), Taiwanese children (27).

The molecular mechanism of its lower secretion in obese people is not fully understood: its down-regulation may be induced by insulin resistance or its secretion may be suppressed by high plasmatic levels of TNF α and IL-6 (18).

In adults, a strong correlation of adiponectin to insulin-resistance biomarkers has been demonstrated and hypo adiponectinemia was associated to progression to type 2 diabetes (17, 28-31). A negative correlation between plasmatic levels of adiponectin and insulin-resistance was also demonstrated in pediatric populations: Taiwanese children (27), Hispanic children (32), Latino children and adolescents (33).

Leptin, primarily adipose tissue-derived protein product of the obesity (*OB*) gene, originally identified as an important regulator of energy metabolism, is a multifunctional polypeptide which may be associated with the occurrence of insulin resistance and diabetes in humans.

Plasma levels of leptin showed positive correlations with BMI, WC and adipose tissue proportion in various adult populations (34-36). In children, these correlations are demonstrated in a limited number of studies and for smaller groups (25,37). Plasmatic levels of leptin seem

to be a better predictor of future weight gain in prospective studies (38-40).

Although *in vitro* and animal models studies have demonstrated the insulin-sensitizing effects of leptin (41-43) hyperleptinemia associated to obesity is associated in clinical studies with insulin-resistance in both obese adults and children (34,36,44,45); this apparent discordance can be explained by a state of leptin resistance described in obese persons (46).

Resistin is a protein secreted in human adipose tissue by preadipocytes (47) and mainly by monocytes from the stromal component (48). Resistin acquired initial attention as a potential link between obesity and glucose regulation. In rodents, resistin can induce insulin resistance, while its implication in the control of insulin sensitivity is still a matter of debate in humans. Several clinical studies have demonstrated that plasmatic levels of resistin are positively correlated to BMI (49-54) while others failed to identify its correlation to adiposity (47,55,56).

There are also controversies regarding resistin's correlation to insulin sensitivity in humans: several clinical studies on variable groups (obese or diabetic persons) have demonstrated that resistin is positively correlated to insulin-resistance (53,57), while other population studies disproved these remarks – resistin was not correlated to insulin-resistance in Pima Indians (52) or healthy normal-weight individuals (55).

TNF α , a protein synthesized especially by macrophages, is a cytokine implied in systemic inflammation. It is also expressed in adipocytes and is associated with obesity (58), adipocytes cell volume (59), and inhibition of glucose uptake in adipocytes from lean individuals (58). TNF α is overproduced in adipose tissue of several rodent models of obesity and has a key role in the pathogenesis of insulin resistance in these species. However, its actual involvement in glucose metabolism disorders in humans remains controversial.

IL-6 production by human adipose tissue increases during obesity. It may induce hepatic C reactive protein synthesis and may promote the onset of cardiovascular complications.

Both TNF- α and IL-6 can alter insulin sensitivity by triggering different key steps in the insulin signaling pathway.

In vitro studies have shown that TNF α and IL-6 have pivotal roles in insulin resistance, acting directly at the insulin receptor (60,61). Both

TNF α and IL-6 are positively related to adiposity, particularly visceral fat, and correlate with insulin resistance and other CVD risk factors in adults (60-64).

In children, data regarding IL-6 and TNF α plasmatic levels are sparse. Levels of these inflammatory markers seem to decrease with increasing age (65) and this can become a confounding factor in interpreting results in obese pediatric population.

Although *in vitro* and animal models studies have indicated a direct relationship between plasmatic TNF α and the degree of adiposity, *in vivo* studies have shown that mRNA expression for TNF α in adipose tissue is not directly dependent to BMI (66); leptin and other adipokines overexpressed in obese persons seem to stimulate TNF α production in the monocyte-macrophage system. This may be an explanation for the paucity of clinical data regarding its role in metabolic complications associated to obesity.

The adipocytokines' plasmatic levels and their correlations to classical metabolic and cardiovascular risk factors are far to be defined in adults and even less in children. \square

OBJECTIVE

The aim of this study is to define the adipocytokines' plasmatic profile in a study group of obese children and adolescents compared to normal-weight controls and to identify possible correlations to classical metabolic and insulin-resistance risk factors. \square

MATERIAL AND METHODS

Study groups

This was a case-control observational study; we analyzed 38 obese children and adolescents (BMI above 95th percentile for age and sex - according to CDC - "Center of disease control" growth charts (67), evaluated for possible metabolic and cardiovascular complications over a six months period in the Pediatric Endocrinology Department of the "C.I.Parhon" National Institute of Endocrinology. We excluded from analysis children and adolescents with primary hypothyroidism (TSH values above 10 μ UI/ml), children with Cushing syndrome, female adolescents with polycystic ovary syndrome. We did not examine patients with syndrome obesity (as Down syndrome or Prader Willi syndrome) or patients treated with insulin sensitizers like metformin.

The control group comprised 24 healthy age-matched normal weight (BMI between 5th and 85th percentiles for age and sex) children and adolescents, with similar pubertal status as the study group.

The study was conducted according to the Declaration of Helsinki, and the study protocol was approved by the Ethical Committee of "C.I.Parhon" National Institute of Endocrinology". Written informed consent was obtained from the parents and informed assent from the children and adolescents.

Anthropometrical measures

Children were evaluated in the morning, after 12 hours of fasting, dressed in light clothes and without shoes; we measured height, weight, waist circumference (WC) and blood pressure (BP); the pubertal status according to Tanner criteria was noted.

Height was measured using a Harpenden stadiometer: an average of 3 determinations at 5 minutes intervals was recorded; body weight was measured with a balance scale to the nearest 0.1 kg and waist circumference was measured with a tape just above the superior iliac crest at the end of normal expiration, according to U.S. Third National Health and Nutrition Examination Survey" recommendations.

Adiposity was evaluated by BMI (calculated as the weight in kilograms divided by the square of the height in meters). BMI variability with age and sex was adjusted using Z-score (standard deviation score) for BMI – we used for automatic calculation an on-line software application based on CDC growth charts (68).

Blood pressure was measured twice daily (in the morning and in the evening) with an appropriate sized cuff and after at least 10 minutes resting in the supine position; the highest systolic and diastolic value respectively were used for analysis.

Paraclinical measurements

We obtained a blood sample after a 12 h overnight fast. Lipid profile (total cholesterol, LDL-cholesterol and HDL-cholesterol, triglyceride), glucose, insulin and adipocytokines levels (adiponectin, leptin, resistin, TNF α and IL-6) were determined.

Plasma insulin was measured using RIA method (Radim, Italia, sensitivity = 1 μ U/ml) on a Gamma-Counter; plasma adiponectin, leptin,

resistin, TNF α and IL-6 were measured by the ELISA Quantikine Human Adiponectin (R&D System, sensitivity = 0.891 ng/ml), Quantikine Human Leptin (R&D System, minimum detectable dose = 7.8 pg/ml), Quantikine Human Resistin (R&D System, sensitivity = 0.026 ng/ml), Quantikine Human TNF α (R&D System, sensitivity = 1.6 pg/mL), Quantikine Human IL-6 (R&D System, minimum detectable dose = 0.039 pg/mL).

For insulin sensitivity assessment we used:

- Homeostatic model index - HOMA-index, calculated as: Fasting glycemia (mg/dl) * fasting insulinemia (μ U/mL) /405
- IRS-score ("insulin-resistance syndrome-summary score") – calculated by adding the quartile ranks from the distribution of systolic BP, TG, HDL-C and insulin levels of each subject.

Statistical analysis

Data were expressed as mean \pm SD for quantitative variables.

Independent t-test student was used to compare differences between variables in the two groups. Bivariate correlations were evaluated with Pearson's coefficient.

We also used stepwise multivariate regression models using either adiponectin, leptin, resistin, TNF α or IL-6 as a dependent variable and including Z-score for BMI, WC, systolic and diastolic BP, metabolic and insulin resistance biomarkers as independent variables. All analyzes were performed using SPSS 16.0 version (SPSS, Chicago, IL, USA). A $p < 0.05$ was considered statistical significant and the confidence interval was 95%. \square

OUTCOMES

There were 38 children and adolescents in the study group (17 girls and 21 boys), aged 13.51 ± 2.31 years; the control group comprised 24 children and adolescents (12 girls, 12 boys), aged 14.27 ± 2.36 years.

Body mass index (BMI), Z-score for BMI, waist circumference (WC), systolic blood pressure and diastolic blood pressure were significantly higher in the study group compared to the control group (Table 1).

As for metabolic and biochemical profile there were statistical significant differences between groups regarding plasmatic levels of triglycerides and the paraclinical markers of hy-

perinsulinism (fasting insulinemia, HOMA-index and IRS) (Table 2).

Plasmatic levels of leptin, resistin and IL-6 were significantly higher in the obese group compared to control group, while plasmatic levels of adiponectin were significantly lower in obese children compared to normal-weight ones (Table 3, Figure 1).

TNF α was also higher in obese children and adolescents but the difference did not reach statistical significance (Table 3).

Univariate regression analysis revealed several significantly correlations between studied adipocytokines and clinical, anthropometric, metabolic parameters and insulin resistance markers (Table 4).

- adiponectin was positively correlated with HDL-cholesterol and negatively correlated with: Z-score for BMI, WC, systolic and diastolic BP, plasmatic triglycerides and IRS;
- leptin was positively correlated with: Z-score for BMI, WC, systolic and diastolic BP, with HOMA-index and fasting insulinemia;
- resistin was positively correlated with Z-score for BMI, with systolic and diastolic BP, with plasmatic levels of leptin and IL-6.
- IL-6 was positively correlated with Z-score for BMI, with systolic and diastolic BP and IRS.

We also found positive correlations between leptin and resistin ($r = 0.444$, $p < 0.001$) and between IL-6 and resistin ($r = 0.307$, $p = 0.015$) and a weak negative but significant correlation between adiponectin and leptin ($r = -0.267$, $p = 0.036$).

TNF α was the only cytokine with no correlation to any of the clinical, metabolic or insulin resistance markers; it had a weak, but significant correlation to IL-6 (Table 4).

Stepwise multiple regression analysis finally indicated that:

- adiponectin was negatively and significantly correlated with WC ($r = -0.463$, $F = 10.078$, $p = 0.003$) (Figure 2);
- leptin was positively and significantly related to WC (Figure 3A), diastolic blood pressure, fasting insulinemia (Figure 3B) and resistin (for this model coefficient of regression was $r = 0.775$, $F = 15.008$, $p < 0.001$);
- resistin was positively related to leptin (Figure 4A) and IL-6 (Figure 4B) ($r = 0.499$, $F = 9.8$, $p < 0.001$);
- IL-6 was positively and significantly related to diastolic blood pressure ($r = 0.333$, $F = 7.467$, $p = 0.008$).

Parameter	Obese (n=38)	Controls (n=24)	p
Age	13.51±2.31	14.27±2.36	0.22
BMI	30.61±3.96	19.2±2.91	<0.001
Z-score for BMI	2.1 ± 0.21	-0.2±1.1	<0.001
WC	103.13±9.48	82.05±11.81	<0.001
Systolic BP	126.71±12.48	102.29±8.96	<0.001
Diastolic BP	78.55±9.92	60.62±7.27	<0.001

TABLE 1. Clinical and anthropometrical parameters of the studied groups.

Parameter	Obese (n=38)	Controls (n= 24)	p
A jeun glicemia	83.04±9.18	83.54±7.1	0.82
Total cholesterol	170±34.52	168.47±25.29	0.85
LDL-cholesterol	100.55±29.55	115.67±31.70	0.12
HDL-cholesterol	48.62±9.49	51.35±10.10	0.38
Tryglicerides	106.19±59.03	76.07±36.04	0.028
Insulinemia	28.07±11.74	19.76±7.71	0.03
HOMA-index	5.79±2.64	4.13±1.85	0.09
IRS	10.89±2.35	7.83±1.90	<0.001

TABLE 2. Biochemical and metabolic profile of the studied groups.

Parameter	Obese (n=38)	Controls (n= 24)	p
Adiponectin ($\mu\text{g/ml}$)	9.05±4.61	15.93±9.24	<0.001
Leptin (ng/ml)	42.42±22.58	14.40±14.49	<0.001
TNF α (pg/ml)	3.18±2.19	2.86±2.74	0.618
IL-6 (pg/ml)	2.66±2.87	0.89±1.16	0.006
Resistin (ng/ml)	9.69±3.47	7.92±2.13	0.029

TABLE 3. Adipocytokines' plasmatic profile in the studied groups.

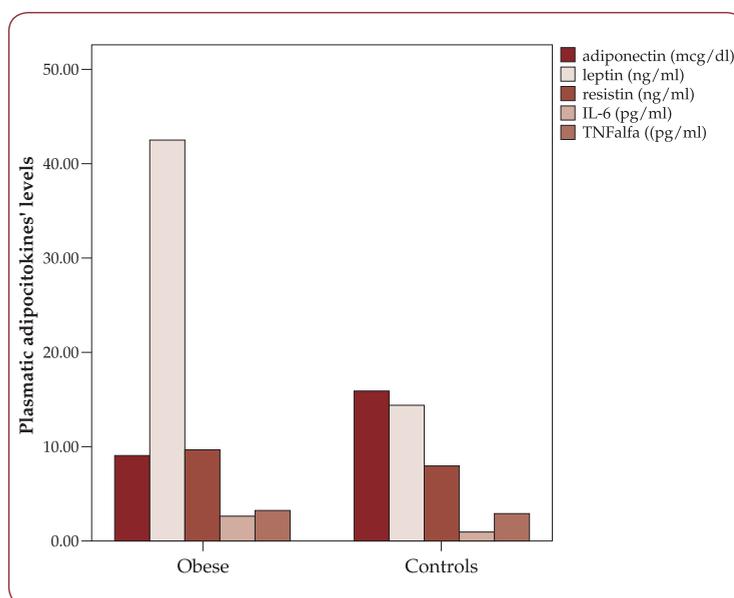


FIGURE 1. Adipocytokines' plasmatic profiles in the studied groups.

The adipocytokines' plasmatic profile was not different according to pubertal stages, and the only significant difference between sexes was those of leptin plasmatic levels (40.29 ± 28.23 pg/ml in females versus 23.91 ± 16.56 pg/ml in males, $p = 0.006$). □

CONCLUSIONS

In this study, we showed that biomarkers of an increased risk of adverse CV outcomes are al-

Profiles	Adiponectin	Leptin	Resistin	TNF α	IL-6
Z score for BMI	r = - 0.557	r = 0.631	r = 0.321	Ns	r = 0.332
	p <0.001	p <0.001	p = 0.011		p = 0.008
WC	r = - 0.513	r=0.599	Ns	Ns	ns
	p <0.001	p <0.001			
Syst BP	r = - 0.393	r = 0.371	r = 0.264	Ns	r = 0.327
	p = 0.02	p = 0.003	p = 0.038		p = 0.010
Diast BP	r = -0.431	r = 0.461	r = 0.319	Ns	r = 0.333
	p <0.001	p <0.001	p = 0.011		p = 0.008
Tryglicerides	r = -0.26	Ns	Ns	Ns	Ns
	p = 0.041				
HDLchol	r = 0.472	ns	ns	ns	ns
	p = 0.001				
insulinemia	ns	r = 0.461	ns	ns	Ns
		p <0.001			
HOMA-index	ns	r = 0.407	ns	ns	ns
		p = 0.001			
IRS	r = -0.432	ns	ns	Ns	r = 0.275
	p <0.001				p = 0.031
Adiponectin	ns	r = -0.267	ns	ns	Ns
		p = 0.036			
Leptin	r = -0.267	Ns	r = 0.444	ns	ns
	p = 0.036		p <0.001		
Resistin	ns	r = 0.444	Ns	ns	r = 0.307
		p <0.001			p = 0.015
IL-6	ns	ns	r = 0.307	r = 0.273	ns
			p = 0.015	p = 0.032	

TABLE 4. Correlation between metabolic parameters and plasmatic adipocytokines.

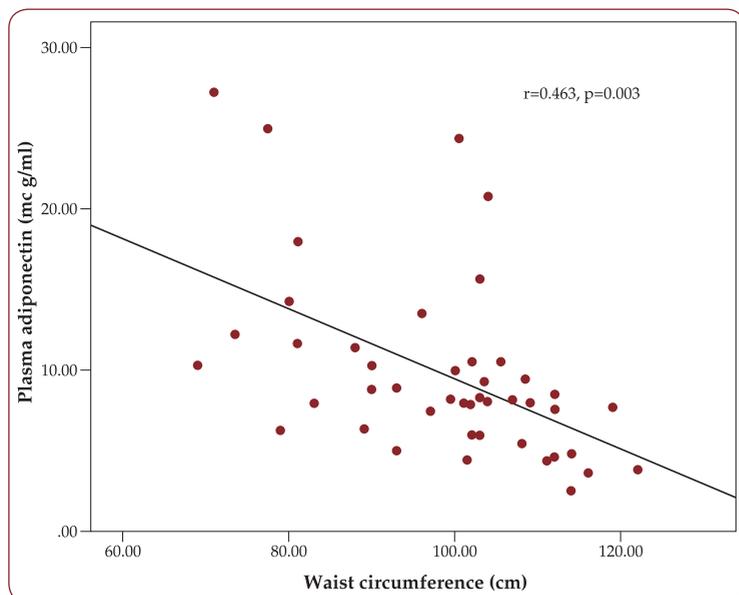


FIGURE 2. Correlation between plasma adiponectin levels and waist circumference.

ready altered in obese children and adolescent: plasmatic levels of HDL-cholesterol, triglycerides and insulin-resistance biomarkers. Not only classical biomarkers of metabolic and CV risk are altered, but also the adipokines' plasmatic profile showed a parallel variation with the degree of obesity.

Excepting TNF α all the other adipocytokines showed significant correlations (negative for adiponectin and positive for others) with clinical biomarkers of obesity and its complications: Z-score for BMI, systolic and diastolic blood pressure.

Adiponectin and leptin were also negatively and respectively positively related to central adiposity quantified by waist circumference.

The only significant correlation between adipokines' plasmatic levels and lipids' profile was found for adiponectin, which positively correlated to HDL-cholesterol and negatively correlated to plasmatic triglycerides; those correlations disappeared in multivariate analysis, being explained by central adiposity.

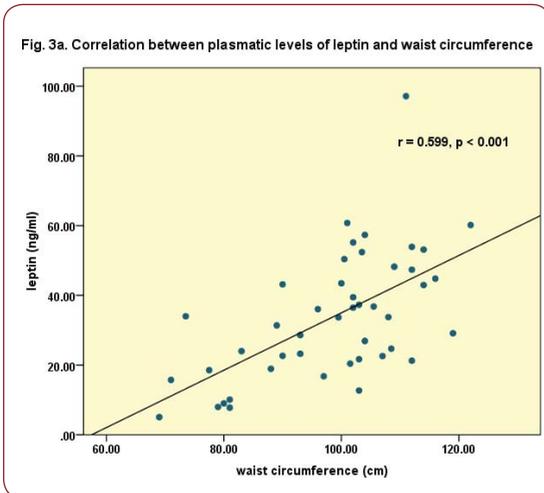


FIGURE 3A. Correlation between plasmatic levels of leptin and waist circumference.

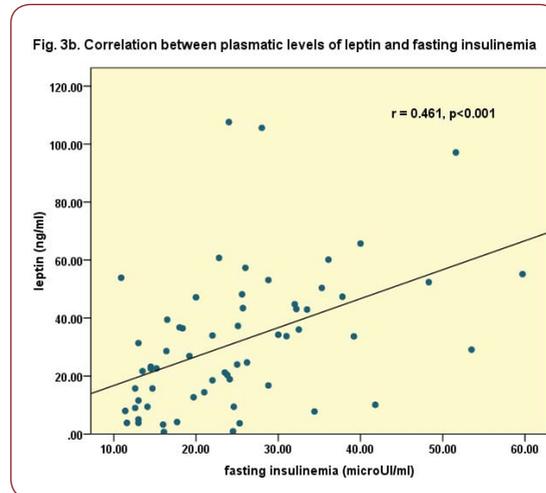


FIGURE 3B. Correlation between plasmatic levels of leptin and fasting insulinemia.

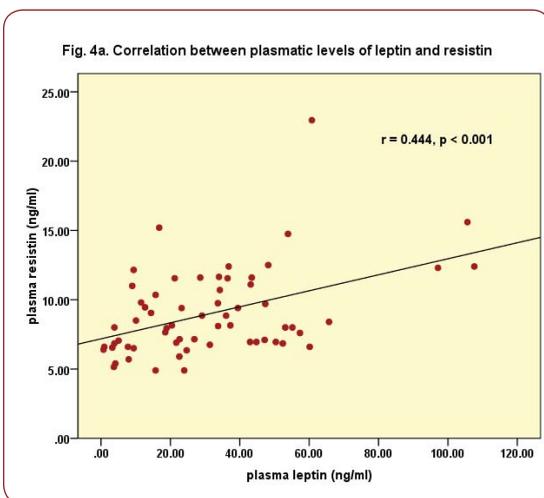


FIGURE 4A. Correlation between plasmatic levels of leptin and resistin.

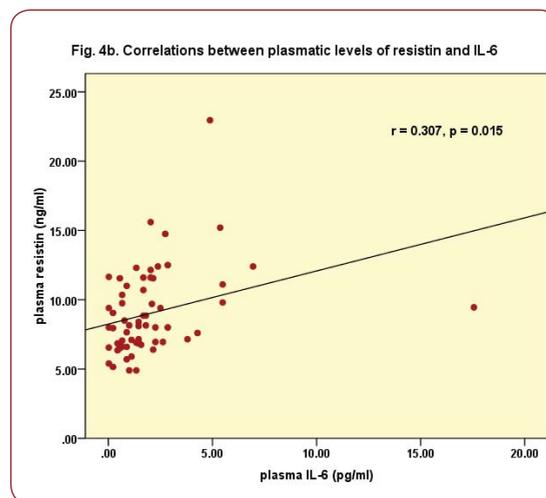


FIGURE 4B. Correlation between plasmatic levels of resistin and IL-6.

Although in univariate analysis adiponectin, leptin and IL-6 were correlated to different paraclinical markers of insulin resistance, in multivariate analysis the only adipokine positively and significantly correlated to insulin resistance was leptin; interestingly, plasmatic levels of resistin showed no correlations with any of the paraclinical markers of the insulin resistance.

Others interesting findings were correlations between plasmatic adipokines' profiles: leptin correlated positively with resistin independent to adiposity degree, resistin was also positively

correlated to IL-6 (independent to Z-score for BMI or WC), adiponectin was negatively correlated to leptin, but the correlation was partially explained by the parallel variation with central adiposity.

Simple plasmatic determination of TNF α was not a marker of the degree of obesity or its metabolic complications in our study.

Adipocytokines' plasmatic profile is a good marker of central obesity in children and a possible marker of future metabolic and cardiovascular complications.

REFERENCES

- Ogden CL, Carroll MD, Curtin LR, et al. – Prevalence of High Body Mass Index in US Children and Adolescents, 2007-2008. *JAMA* 2010;303:242-9
- www.easo.org/childhood-obesity-cotf/facts-a-statistics
- Kosti RI, Panagiotakos DB – The epidemic of obesity in children and adolescents in the world. *Cent Eur J Public Health* 2006;14:151-159
- Korsten-Reck U, Kromeyer-Hauschild K, Korsten K, et al. – Frequency of secondary dyslipidemia in obese children. *Vasc Health Risk Manag* 2008; 4:1089-94
- Pastucha D, Horáková D, Janoutová G, et al. – Epidemiology of the metabolic syndrome and possibility for its prevention by physical activities. *Epidemiol Mikrobiol Imunol* 2007;56:181-5
- Sesé MA, Jiménez-Pavón D, Gilbert CC, et al. – HELENA Study Group. Eating behaviour, insulin resistance and cluster of metabolic risk factors in European adolescents. The HELENA study. *Appetite* 2012; 59:140-7. Epub 2012, Apr 21
- Angelopoulos PD, Milionis HJ, Moschonis G, et al. – Relations between obesity and hypertension: preliminary data from a cross-sectional study in primary schoolchildren: the children study. *Eur J Clin Nutr* 2006;60:1226-34
- Berg AH, Combs TP, Du X, et al. – The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med* 2001;7:947-53
- Yamauchi T, Kamon J, Minokoshi Y, et al. – Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 2002; 8:1288-95
- Arsal N, Erdur B, Aydin A – Hormones and Cytokines in Childhood Obesity. *Indian Pediatrics* 2010;47:829-39
- Ouchi N, Kihara S, Arita Y, et al. – Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation* 1999; 100:2473-6
- Kato H, Kashiwagi H, Shiraga M, et al. – Adiponectin acts as an endogenous antithrombotic factor. *Arterioscler Thromb Vasc Biol* 2006; 26:224-230.
- Okamoto Y, Kihara S, Ouchi N, et al. – Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. *Circulation* 2002; 106:2767-70
- Shimada K, Miyazaki T, Daida H – Adiponectin and atherosclerotic disease. *Clin Chim Acta* 2004;344:1-12
- Matsuo Y, Imanishi T, Kuroi A, et al. – Effects of plasma adiponectin levels on the number and function of endothelial progenitor cells in patients with coronary artery disease. *Circ J* 2007; 71:1376-82
- Ouchi N, Ohishi M, Kihara S, et al. – Association of hypoadiponectinemia with impaired vasoreactivity. *Hypertension* 2003; 42:231-4
- Weyer C, Funahashi T, Tanaka S, et al. – Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* 2001; 86:1930-1935
- Arita Y, Kihara S, Ouchi N, et al. – Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 1999; 257:79-83
- Bruun JM, Lihn AS, Verdich C, et al. – Regulation of adiponectin by adipose tissue-derived cytokines: in vivo and in vitro investigations in humans. *Am J Physiol Endocrinol Metab* 2003; 285:E527-33
- Cnop M, Havel PJ, Utzschneider KM, et al. – Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia* 2003; 46:459-69
- Hu E, Liang P, Spiegelman BM – AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem* 1996; 271:10697-703
- Kern PA, DiGregorio GB, Lu T, et al. – Adiponectin expression from human adipose tissue: relationship to obesity, insulin resistance and TNF alpha expression. *Diabetes* 2003; 52(suppl 1):A329
- Kwon K, Jung SH, Choi C, et al. – Reciprocal association between visceral obesity and adiponectin: in healthy premenopausal women. *Int J Cardiol* 2005;101:385-90
- Yang WS, Lee WJ, Funahashi T, et al. – Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. *J Clin Endocrinol Metab.* 2001; 86:3815-9
- Stefan N, Bunt J, Salbe AD, et al. – Adiponectin Concentrations in Children: Relationships with Obesity and Insulinemia. *JCEM* 2002; 87:4652-4656
- Asayama K, Hayashibe H, Dobashi K, et al. – Decrease in serum adiponectin level due to obesity and visceral fat accumulation in children. *Obes Res* 2003; 11:1072-9
- Chu NF, Shen MH, Wu DM, et al. – Relationship between Plasma Adiponectin Levels and Metabolic Risk Profiles in Taiwanese Children. *Obesity Research* 2005; 13:2014-2020
- Spranger J, Kroke A, Mohlig M, et al. – Adiponectin and protection against type 2 diabetes mellitus. *Lancet* 2003; 361:226-228
- Snehalatha C, Ramachandran A, Satyavani K, et al. – Plasma leptin is not associated with insulin resistance and proinsulin in non-diabetic South Asian Indians. *Diabet Med* 1998; 15;6:480-4
- Duncan BB, Schmidt MI, Pankow JS, et al. – Adiponectin and the development of type 2 diabetes: the atherosclerosis risk in communities study. *Diabetes* 2004; 53:2473-8
- Stefan N, Vozarova B, Funahashi T, et al. – Plasma adiponectin concentration is associated with skeletal muscle insulin receptor tyrosine phosphorylation, and low plasma concentration precedes a decrease in whole-body insulin sensitivity in humans. *Diabetes* 2002; 51:1884-8
- Butte NF, Comuzzie AG, Cai G, et al. – Genetic and environmental factors influencing fasting serum adiponectin in Hispanic children. *J Clin Endocrinol Metab* 2005;90:4170-6
- Shaibi GQ, Cruz ML, Weigensberg MJ, et al. – Adiponectin independently predicts metabolic syndrome in overweight Latino youth. *J Clin Endocrinol Metab* 2007; 92:1809-1813
- Zimmet P, Hodge A, Nicolson M, et al. – Serum leptin concentration, obesity, and insulin resistance in Western Samoans: cross sectional study. *BMJ* 1996; 313:965
- Liuzzi A, Savia G, Tagliaferri M, et al. – Serum leptin concentration in moderate and severe obesity: relationship with clinical, anthropometric and metabolic factors. *Intern J Obes* 1999; 23:1066-1073
- Ganji V, Kafai MR, McCarthy E – Serum leptin concentrations are not related to dietary patterns but are related to sex, age, body mass index, serum triacylglycerol, serum insulin, and plasma glucose in the US population. *Nutr & Metab* 2009; 6:3
- Yamborisut U, Riabroy U, Phonrat B, et al. – Serum leptin levels and body composition in obese Thai children. *The Southeast Asian Journal of Tropical Medicine and Public Health* 2009; 40: 544-552
- Fleisch AF, Agarwal N, Roberts MD, et al. – Influence of Serum Leptin on Weight and Body Fat Growth in Children at High Risk for Adult Obesity. *J Clin Endocrinol Metab* 2007; 92: 948-954
- Barbeau P, Gutin B, Litaker MS, et al. – Influence of Physical Training on Plasma Leptin in Obese Youths. *Canadian Journal of Applied Physiology* 2003; 28:382-96
- Johnson MS, Huang TTK, Figueroa-Colon R, et al. – Influence of Leptin on Changes in Body Fat during Growth in African American and White Children. *Obes Res* 2001; 9:593-598
- Kamohara S, Burcelin R, Halaas JL, et al. – Acute stimulation of glucose

- metabolism in mice by leptin treatment. *Nature* 1997; 389:374-7
42. Wang JL, Chinooskong N, Scully S, et al. – Differential effects of leptin in regulation of tissue glucose utilization in vivo. *Endocrinology* 1999; 140:2117-24
 43. Rossetti L, Massillon D, Barzilai N, et al. – Short term effects of leptin on hepatic gluconeogenesis and in vivo insulin action. *J Biol Chem* 1997; 272:27758-63
 44. Zimmet P, Collins VR, de Courten MP, et al. – Is there a relationship between leptin and insulin sensitivity independent of obesity? A population-based study in the Indian Ocean nation of Mauritius. *Intern J Obes* 1998; 2:171-177
 45. Chu NF, Chang JB, Shieh SM – Plasma Leptin, Fatty Acids, and Tumor Necrosis Factor-Receptor and Insulin Resistance in Children. *Obes Res*. 2003; 11:532-540
 46. Considine RV, Sinha MK, Heiman ML, et al. – Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 1996;334:292-295
 47. Janke J, Engeli S, Gorzelnik K, et al. – Resistin gene expression in human adipocytes is not related to insulin resistance. *Obes Res* 2002; 10:1-5
 48. Kaser S, Kaser A, Sandhofer A, et al. – Resistin messenger RNA expression is increased by proinflammatory cytokines in vitro. *Biochem Biophys Res Commun* 2003; 309:286-290
 49. Yannakoulia M, Yiannakouris N, Bluher S, et al. – Body fat mass and macronutrient intake in relation to circulating soluble leptin receptor, free leptin index, adiponectin, and resistin concentrations in healthy humans. *J Clin Endocrinol Metab* 2003;88:1730-6
 50. Azuma K, Katsukawa F, Oguchi S, et al. – Correlation between serum resistin level and adiposity in obese individuals. *Obes Res* 2003; 11:997-1001
 51. Degawa-Yamauchi M, Bovenkerk JE, Juliar BE – Serum resistin (FIZZ3) protein is increased in obese humans. *J Clin Endocrinol Metab* 2003; 88: 5452-5455
 52. Volarova de Courten B, Degawa-Yamauchi M, Considine RV, et al. – High serum resistin is associated with an increase in adiposity but not a worsening of insulin resistance in Pima Indians. *Diabetes* 2004; 53:1279-1284
 53. Silha JV, Krsek M, Skrha JV, et al. – Plasma resistin, adiponectin and leptin levels in lean and obese subjects: correlations with insulin resistance. *Eur J Endocrinol* 2003; 149:331-5
 54. Savage DB, Sewter CP, Klenk ES, et al. – Resistin/Fizz3 expression in relation to obesity and peroxisome proliferator-activated receptor-gamma action in humans. *Diabetes* 2001;50:2199-202
 55. Lee JH, Chan JL, Yiannakouris N, et al. – Circulating resistin levels are not associated with obesity or insulin resistance in humans and are not regulated by fasting or leptin administration: cross-sectional and interventional studies in normal, insulin-resistant, and diabetic subjects. *J Clin Endocrinol Metab* 2003;88:4848-4856
 56. Heilbronn LK, Rood J, Janderoova L, et al. – Relationship between serum resistin concentrations and insulin resistance in nonobese, obese, and obese diabetic subjects. *J Clin Endocrinol Metab* 2003; 89:1844-8
 57. Youn BS, Yu KY, Park HJ, et al. – Plasma resistin concentrations measured by enzyme-linked immunosorbent assay using a newly developed monoclonal antibody are elevated in individuals with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2004; 89: 150-156
 58. Winkler G, Kiss S, Keszthelyi L, et al. – Expression of tumor necrosis factor (TNF)-alpha protein in the subcutaneous and visceral adipose tissue in correlation with adipocyte cell volume, serum TNF-alpha, soluble serum TNF receptor-2 concentrations and C-peptide level. *Eur J Endocrinol* 2003; 149:129-35
 59. Good M, Newell FM, Haupt LM, et al. – TNF and TNF receptor expression and insulin sensitivity in human omental and subcutaneous adipose tissue—influence of BMI and adipose distribution. *Diab Vasc Dis Res* 2006; 3:26-33
 60. Hotamisligil GS, Shargill NS, Spiegelman BM – Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 1993; 259:87-91
 61. Mendall MA, Patel P, Asante M, et al. – Relation of serum cytokine concentrations to cardiovascular risk factors and coronary heart disease. *Heart* 1997; 78:273-277
 62. Shai I, Schulze MB, Manson JE, et al. – A prospective study of soluble tumor necrosis factor-alpha receptor II (sTNF-RII) and risk of coronary heart disease among women with type 2 diabetes. *Diabetes Care* 2005; 28:1376-1382
 63. Liu S, Tinker L, Song Y, et al. – A prospective study of inflammatory cytokines and diabetes mellitus in a multiethnic cohort of postmenopausal women. *Arch Intern Med* 2007; 167:1676-1685
 64. Le Roith D, Zick Y – Recent advances in our understanding of insulin action and insulin resistance. *Diabetes Care* 2001; 24:588-597
 65. Balagopal P, de Ferranti SD, Cook S, et al. – Nontraditional Risk Factors and Biomarkers for Cardiovascular Disease: Mechanistic, Research, and Clinical Considerations for Youth: A Scientific Statement From the American Heart Association. *Circulation* 2011; 123:2749-2769
 66. Koistinen HA, Bastard JP, Dusserre E, et al. – Subcutaneous adipose tissue expression of tumour necrosis factor-alpha is not associated with whole body insulin resistance in obese nondiabetic or in type-2 diabetic subjects. *Eur J Clin Invest* 2000; 30: 302-310
 67. www.cdc.gov/growthcharts
 68. <http://stokes.chop.edu/web/zscore>.