Adipocytokine Profile and Insulin Resistance in Childhood Obesity

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\textbf{ABSTRACT}

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

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

\textbf{Material and Methods:} A case-control study comparing a study group of 38 obese children and adolescents (age 13.5±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\textalpha were measured using ELISA method.

\textbf{Outcomes:} Serum levels of leptin, resistin and IL-6 were significantly higher (42.42±22.58 ng/ml versus 14.4±14.49 ng/ml, \(p<0.001\); 9.69±3.47 ng/ml versus 7.92±2.13ng/ml, \(p=0.029\) and 2.66 ±2.87 pg/ml versus 0.89 ± 1.16 pg/ml, \(p=0.006\) respectively), while adiponectin levels were significantly lower (9.05±4.61 \(\mu\)g/ml versus 15.93±9.24 \(\mu\)g/ml, \(p<0.001\) ) in the obese group compared to control group. TNF\textalpha 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 insulinenia and resistin (\(r=0.775, p<0.001\)); resistin was positively related to leptin and IL-6 (\(r=0.499, p=0.001\)).
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 TNFα and IL-6 (18).

In adults, a strong correlation of adiponectin to insulin-resistance biomarkers has been demonstrated and hypoadiponectinemia 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 ef-
facts of leptin (41-43) hyperleptinemia associ-
ated to obesity is associated in clinical studies
with insulin-resistance in both obese adults and
children (34,36,44,45); this apparent discord-
ance can be explained by a state of leptin re-
sistance described in obese persons (46).

Resistin is a protein secreted in human
adipose tissue by preadipocytes (47) and main-
ly by monocytes from the stromal component
(48). Resistin acquired initial attention as a po-
tential link between obesity and glucose regula-
tion. In rodents, resistin can induce insulin re-
sistance, while its implication in the control of
insulin sensitivity is still a matter of debate in
humans. Several clinical studies have demon-
strated that plasmatic levels of resistin are posi-
tively correlated to BMI (49-54) while others
failed to identify its correlation to adiposity
(47,55,56).

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

TNFα, 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 up-
take in adipocytes from lean individuals (58).
TNFα is overproduced in adipose tissue of sev-
eral 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 re-
 mains 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-a and IL-6 can alter insulin sensi-
tivity by triggering different key steps in the insu-
lin signaling pathway.

In vitro studies have shown that TNFα and
IL-6 have pivotal roles in insulin resistance, act-
ing directly at the insulin receptor (60,61). Both
TNFα and IL-6 are positively related to adipos-
ity, particularly visceral fat, and correlate with
insulin resistance and other CVD risk factors in
adults (60-64).

In children, data regarding IL-6 and TNFα
plasmatic levels are sparse. Levels of these in-
flammatory markers seem to decrease with in-
creasing age (65) and this can become a con-
founding factor in interpreting results in obese
pediatric population.

Although in vitro and animal models studies
have indicated a direct relationship between
plasmatic TNFα and the degree of adiposity, in
vivo studies have shown that mARN expression
for TNFα in adipose tissue is not directly depen-
dent to BMI (66); leptin and other adipokines
overexpressed in obese persons seem to stimu-
late TNFα production in the monocyte-macro-
phage 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 car-
diovascular risk factors are far to be defined in
adults and even less in children.

OBJECTIVE

The aim of this study is to define the adipocy-
tokines’ 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.

MATERIAL AND METHODS

Study groups

This was a case–control observational study;
we analyzed 38 obese children and adoles-
cents (BMI above 95th percentile for age and
sex - according to CDC - “Center of disease
control” growth charts (67), evaluated for possi-
ble metabolic and cardiovascular complications
over a six months period in the Pediatric
Endocrinology Department of the “C.I.Parhon”
National Institute of Endocrinology. We exclud-
ed from analysis children and adolescents with
primary hypothyroidism (TSH values above 10
μUI/ml), children with Cushing syndrome, fem-
ale adolescents with polycystic ovary syn-
drome. We did not examine patients with syn-
Adipocytokine profile and insulin resistance in childhood obesity

112

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drome 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, TNFα and IL-6) were determined.

Plasma insulin was measured using RIA method (Radim, Italia, sensitivity = 1 μU/ml) on a Gamma-Counter; plasma adiponectin, leptin, resistin, TNFα 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 TNFα (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 (μIU/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 ± 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, TNFα 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%.

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 hyperinsulinism (fastinginsulinemia, 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).

TNFα 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).

TNFα was the only cytokine with no correlation to any of the clinical, metabolic or insulin resistance markers; it had a week, 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).

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 already 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 TNFα all the other adipokines 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.

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 TNFα 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.
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