Original article

Adiponectin, IL-10 and metabolic syndrome in obese children and adolescents

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Abstract. The metabolic syndrome (MetS) is a common basis for the development of atherogenic cardiovascular disease. Adiponectin has been demonstrated to be insulin-sensitizing and an anti-atherogenic factor and is considered a key of MetS. It was suggested that IL-10 may be involved in the inflammatory network of MetS in relation to adiponectin. We examined the relationship between adiponectin, IL-10 and MetS in pediatric obese patients. MetS components were assessed in 70 severely obese and 30 non-obese children and adolescents. Serum levels of adiponectin and IL-10 were measured in these subjects. Serum adiponectin levels were significantly lower ($p<0.001$) and levels of IL-10 were significantly higher ($p=0.012$) in obese subjects. MetS was present in 35.71% of obese patients. Patients with MetS showed a borderline significant decrease in serum adiponectin levels and significantly increased IL-10 levels when compared to those without MetS ($p=0.051$ and $p=0.031$, respectively); the differences in adiponectin and IL-10 values were controlled to the effect of BMI. No correlation between adiponectin and IL-10 levels was found. Our obese children showed hypoadiponectin and hyper-IL10 values. MetS was not associated with low IL-10. We probably observe a first phase of the complex mechanism implicated in the development of the MetS in children. (www.actabiomedica.it)

Key words: Adiponectin, IL-10, metabolic syndrome, obesity

Introduction

The metabolic syndrome (MetS), representing a cluster of insulin resistance, glucose intolerance, hypertension, and dyslipidemia, is a common basis for the development of atherogenic cardiovascular disease. The pathophysiology of the MetS has not been elucidated. Adiponectin, an anti-inflammatory protein (1), has been demonstrated to be insulin-sensitizing and an anti-atherogenic factor including coronary spasms, and is considered a key of MetS (2, 3). Serum adiponectin levels are known to decrease with the increase in the number of MetS components (4). However, low plasmatic adiponectin concentrations are found in obese subjects (5).

Interleukin-10 (IL-10) has anti-inflammatory properties and exhibits a protective effect against atherogenesis (6-8). It was shown that adiponectin induces expression of IL-10 in human macrophages (9), and that anti-atherogenic effects of adiponectin are partially mediated by the induction of IL-10. Recently, in an adult population, it was suggested that IL-10 may be involved in the inflammatory network of MetS in relation to adiponectin (10).

In the present study, we examined the relationship between adiponectin, IL-10 and MetS in obese children and adolescents.

Patients

We studied 70 severely obese children and adolescents aged 11.46±3.42 yrs (37 females and 33 males, 20 pre-pubertal and 50 pubertal), with body mass indexes (BMI, calculated as body weight in kilograms divided by height squared in meters) that exceeded the 97th percentile for their age and sex and with BMIz-score>2.5, adjusted for age and sex (11). Subjects were referred to our institute for obesity by their general practitioner or primary care pediatric consultant. Exclusion criteria were any known secondary obesity syndromes, any ongoing medical therapy and concomitant chronic or acute illnesses.

As a control group, 30 non-obese siblings of obese subjects, aged 10.18±3.68 yrs (10 females and 20 males, 16 pre-pubertal and 14 pubertal) were recruited.

Methods

The physical examination of the patients included an evaluation of weight, height, BMI, pubertal stage according to Marshall and Tanner (12-13) and blood pressure measurement.

Fasting blood glucose levels, insulin, total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, adiponectin and IL-10 were measured in all patients.

Serum glucose was measured using the hexokinase - G6P-DH method (Abbott Diagnostics, Abbott Park, IL, USA); intra- and interassay CVs were 1.98% and 2.15%, respectively, at 6.5 mmol/L and 0.65% and 1.51% at 20.3 mmol/L. Total cholesterol was determined by enzymatic method (Abbott Diagnostics, Abbott Park, IL, USA); intra- and interassay CVs were 0.8% and 1.5%, respectively, at 4.5 mmol/L and 0.6% and 1.6% at 3.3 mmol/L. HDL-cholesterol was measured by accelerator selective detergent method (Abbott Diagnostics, Abbott Park, IL, USA), intra- and interassay CVs were 1.4 % and 5.5%, respectively, at 1.45 mmol/L and 2% and 1.4% at 2 mmol/L. Triglyceride concentration was measured by the glycerol phosphatase oxidase method (Abbott Diagnostics, Abbott Park, IL, USA); intra- and interassay CVs were 0.7% and 1.7%, respectively, at 1.34 mmol/L and 0.8% and 2% at 1.66 mmol/L. Serum insulin was determined by a solid-phase, two-site chemiluminescent immunometric assay (Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA); intra- and interassay CVs were 5.5% and 8%, respectively, at 5 μIU/ml and 3.8% and 4.2% at 42 μIU/ml.

An oral glucose tolerance test was performed administering 1.75 g of glucose per kilogram of body weight (maximal dose 75 g). Impaired glucose tolerance was defined as a glucose level greater than 7.8 mmol/L but lower than 11.1 mmol/L after two hours (14).

A triglyceride value exceeding the 95th percentile and a HDL cholesterol value below the 5th percentile for age and sex were considered abnormalities in the fasting levels of lipids (15).

Systolic and diastolic blood pressures were taken twice with the patient in a sitting position. Elevated systolic or diastolic blood pressure was defined as a value exceeding the 95th percentile for age and sex (16).

As previously reported (17) and according to Weiss (18), metabolic syndrome was diagnosed using the criteria modified from those of the National Cholesterol Education Program's Adult Treatment Panel III (NCEP-ATPIII) (19) and the World Health Organization (20). Patients were classified as having MS if they met three or more of the following criteria for age and sex: BMI >97th percentile, triglyceride levels >95th percentile, HDL cholesterol level <5th percentile, systolic and/or diastolic blood pressure >95th percentile, impaired glucose tolerance.

Insulin resistance was calculated using the homeostasis model assessment for insulin resistance (HOMA-IR) (21).

Serum adiponectin was measured through a quantitative sandwich enzyme immunoassay technique (Quantikine Human Adiponectin Immunoassay, R&D System, Minneapolis, USA); intra-assay CVs were 2.5% at 19.8 pg/ml and 4.7% at 143 pg/ml

and inter-assay CVs were 6.8% at 20.5 pg/ml and 6.9% at 157 pg/ml.

IL-10 was measured through a quantitative sandwich enzyme immunoassay technique (Quantikine Human IL-10 Immunoassay, R&D System, Minneapolis, USA); intra-assay CVs were 5.0% at 23.9 pg/ml and 1.7% at 231 pg/ml and inter-assay CVs were 7.3% at 23.2 pg/ml and 5.9% at 228 pg/ml.

The study protocol was approved by the Ethical Committee of our Institution. All the patients and/or their parents gave their informed consent.

Statistical analysis

Continuous variables were described as mean and standard deviation (SD) or median and interquartile range (IQR) and categorical variables were described as counts and percentages.

Comparisons between independent samples were performed with the Mann Whitney U test or the Fisher exact test, for continuous and categorical variables, respectively. For the purpose of our analysis, adiponectin and IL-10 were log-transformed. The Pearson R and its 95% confidence interval (95%CI) were calculated to assess the association of adiponectin and IL-10 (log scale). Moreover, their association was assessed while controlling for BMI in a general linear regression model. The interaction with BMI was tested and excluded.

Stata 10 (StataCorp, College Station, TX, USA) was used for computations. All tests were 2-sided.

Results

The characteristics of the study subjects are shown in Table 1.

Compared with normal-weight children and adolescents, obese patients had higher fasting glucose, insulin, triglycerides and blood pressure and lower HDL cholesterol concentrations (Table 1). Serum adiponectin levels were significantly lower and levels of circulating IL-10 were significantly higher in obese subjects. In both groups, adiponectin and IL-10 levels were not significantly different in males and females (Table 2) or in pubertal and pre-pubertal individuals (Table 3).

The serum levels of adiponectin did not correlate with IL-10 levels (R=-20%; 95%CI: -38%-0%,

Table 1. Clinical characteristics of the study subjects

Characteristics	Normal-weight (n=30)	Severely obese (n=70)	P value
BMI (Kg/m^2)	17.92±2.58	35.14±5.0	<0.001
BMI z-score	-0.31±0.72	2.94±0.40	<0.001
Triglycerides (mmol/L)§	0.66 (0.52-0.83)	1.27 (0.95-1.79)	<0.001
Total cholesterol (mmol/L)	3.85±0.55	4.20±0.90	0.01
HDL-cholesterol (mmol/L)	1.24±0.25	0.99±0.25	<0.001
Systolic pressure (mmHg)	105.28±12.77	125.57±12.88	<0.001
Diastolic pressure (mmHg)	66.39±9.36	75.74±10.56	0.001
Blood glucose (mmol/L)			
Fasting	4.65±0.38	4.90±0.46	0.006
After two hours	5.72±0.86	6.25±1.37	0.004
Fasting insulin (μIU/ml) §	4.70 (3.70-6.40)	18.0 (13.0-26.0)	<0.001
HOMA-IR§	0.96 (0.73-1.42)	3.86 (2.77-5.41)	<0.001
Metabolic syndrome	0/30	25/70 (35.7%)	<0.001*
Adiponectin (μg/ml)§	13.68 (5.60-15.09)	1.25 (0.63-1.96)	<0.001
IL-10 (pg/ml)§	6.14 (1.23-8.66)	11.67 (2.23-25.67)	0.012

§ median (IQR),* Fisher exact test

Table 2. Adiponectin and IL-10 levels in normal-weight and obese subjects according to sex

	Normal-weight			Severely obese		
	Males	Females	p	Males	Females	p
Adiponectin (μg/ml)§	13.66 (5.74-15.34)	13.68 (5.09-14.94)	0.53	1.29 (0.56-1.95)	1.04 (0.67-1.73)	0.91
IL-10 (pg/ml)§	4.74 (0.57-8.61)	6.74 (5.81-10.61)	0.22	11.67 (2.23-31.78)	13.68 (2.80-22.12)	0.90

§ median (IQR)

Table 3. Adiponectin and IL-10 levels in normal-weight and obese subjects according to pubertal status

	Normal-weight			Severely obese		
	Pre-pubertal	Pubertal	p	Pre-pubertal	Pubertal	p
Adiponectin (μg/ml)§	13.68 (5.88-15.08)	13.64 (4.92-15.87)	1	1.13 (0.59-2.6)	1.26 (0.66-1.81)	0.81
IL-10 (pg/ml)§	4.70 (0.57-6.98)	7.40 (4.65-12.54)	0.16	11.41 (5.57-24.02)	13.48 (1.98-25.67)	0.92

§ median (IQR)

P=0.049) and the strength of the association further decreased when accounting for BMI (R=-16%, p=0.115, Figure 1).

The MetS was present in 35.71% of obese patients while no normal weight subjects met the criteria for MetS (p<0.001).

Patients with MetS showed a borderline significant decrease in serum adiponectin levels and significantly increased IL-10 levels when compared to those without MetS (adiponectin: 3.56 (0.73-8.97) *vs* 1.37 (0.98-1.81), p=0.051 and IL-10: 7.19 (1.28-7.76) *vs* 16.55 (4.85-34.65), p=0.031). However, the differences in adiponectin levels completely disappeared when controlling the effect of BMI (adjusted p=0.837), while the differences in IL-10 markedly lost in statistical relevance (adjusted p=0.052).

In subjects with MetS, no correlation between adiponectin and IL-10 was found (R=16%; 95%CI: -25%-52%, P=0.45, Figure 2).

No significant correlation was present between adiponectin and IL-10 and fasting blood glucose, blood glucose levels after 2 hours, fasting insulin, HOMA, HDL-cholesterol, triglycerides, systolic and diastolic blood pressure (Spearman R from 0% to 36%).

Discussion

Obese children are at high risk of adult obesity and childhood obesity provides an independent contribution to the development of adult morbidity (22-24). In particular, obesity plays a central role in the MetS which includes abnormalities in glucose metabolism, hypertension, dyslipidemia, and an increased risk of developing diabetes mellitus and cardiovascular disease (25, 26). The MetS is increasingly recognized in childhood (25, 27, 28).

In our pediatric population MetS is only present in obese subjects. A recent research has demonstrated that adipose tissue produces and secretes various bioactive substances, conceptualised as adipocytokines, and their dysregulation in abdominal or visceral obesity may participate in the development of the MetS (29, 30).

Adiponectin is an adipose-derived protein, with multivalent functions including anti-atherogenic, insulin-sensitizing, lipid-oxidation enhancing and vasodilatory activities (5, 31, 32). The serum adiponectin levels were associated with MetS more clearly and independently than the other inflammatory factors. Therefore, it is possible that decreased plasma concen-

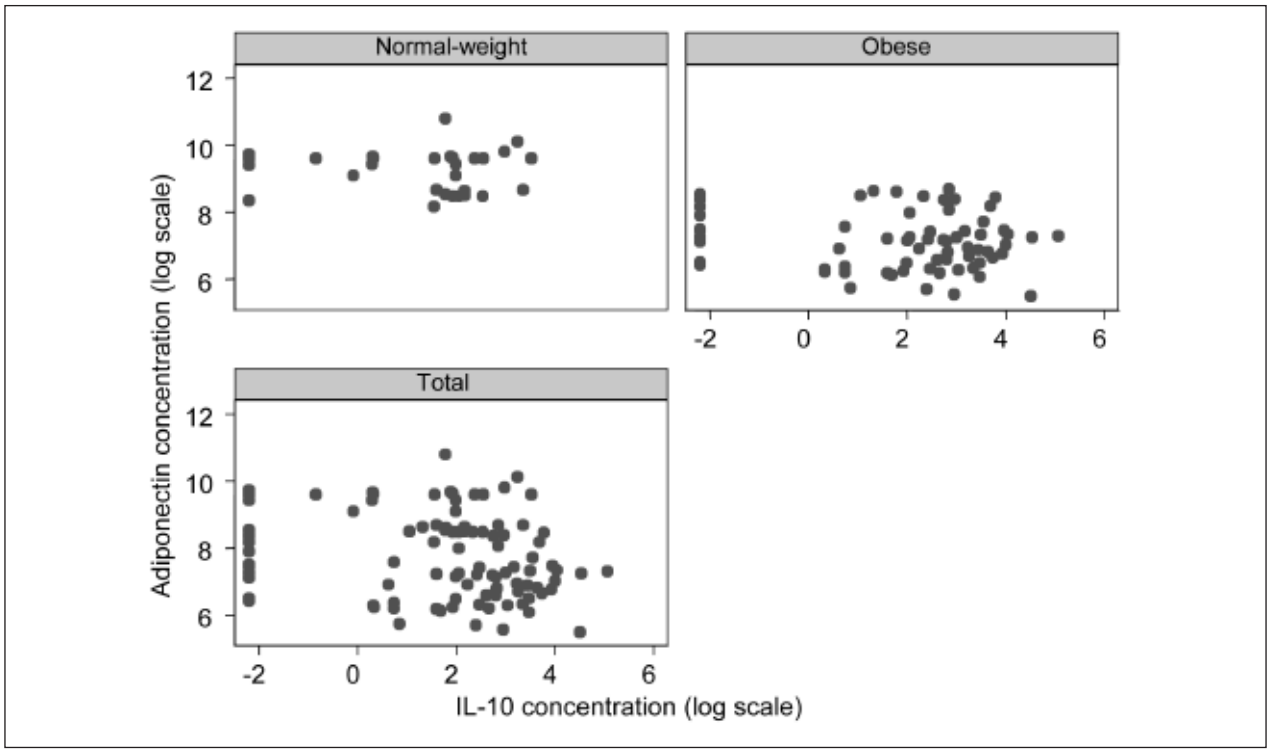

Figure 1. Correlation between log-transformed adiponectin and log-transformed IL-10

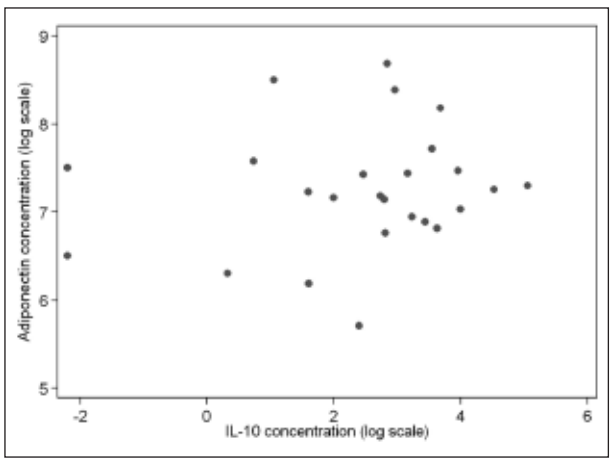

Figure 2. Correlation between log-transformed adiponectin and log-transformed IL-10 in patients with MetS

trations of adiponectin play a significant role in the development of the MetS (33).

Our data in a pediatric population confirm, as reported in adults (5), that hypoadiponectinemia is observed in obese subjects and that serum adiponectin levels were decreased in patients with MetS but this difference disappeared when controlling the effect of BMI.

Recently, it was shown that anti-inflammatory effects of adiponectin may be partially mediated by the induction of IL-10, a potent anti-inflammatory cytokyne (10). *In vivo*, IL-10 most likely exerts its anti-inflammatory effects on the vascular system through the inhibition of leukocyte-endothelial cell interactions and inhibition of proinflammatory cytokines and chemokine production by macrophages or lymphocytes (34).

The mechanism of the link between abdominal obesity and serum adiponectin and IL-10 levels are speculated as follows. Immunohistochemical analysis of adipose tissue demonstrated the presence of macrophages. It has been reported that macrophages are a source of many adipose-derived proteins. The finding of increased numbers of macrophages infiltrating the visceral fat tissue in obese individuals suggests that adipose tissue itself is a source and site of inflammation. IL-10 is a pleiotropic cytokine mainly

derived from T-cells and macrophages in bone marrow, but increased visceral fat may be an alternative source for circulating IL-10 in obese subjects (10).

Esposito et al (35) reported that circulating levels of IL-10 are elevated in obese women and that low levels of IL-10 are associated with the MetS. In our obese patients serum levels of IL-10 were elevated but on the contrary MetS was not associated with low IL-10. As reported (35), the higher IL-10 levels observed in obese patients represent an attempt to inhibit continued proinflammatory cytokine production, which, however, fails in those with a low innate IL-10 production. About three fourths of the differences in IL-10 production in humans are derived from heritable factors (36). Recent studies have demonstrated associations between lower serum IL-10 concentration or production and clinical events (37-39). It is possible that, after the compensatory hyper-IL10 already operative in the pediatric population, children with a reduced capacity of producing IL-10 in adult age presented a progressive decrease of IL-10 and an increased risk for developing type 2 diabetes and cardiovascular disease.

In contrast with the data reported by Esposito (35), in our patients with MetS no correlation between adiponectin and IL-10 was found. Manigrasso et al (40) described that only android obesity is associated with a concomitant reduction of IL-10 and adiponectin and concluded that different fat distribution may be responsible for the decreased levels of this cytochine; in pediatric males and females the difference in body proportions related to puberty influence fat distribution and the different fat distribution with respect to adult age may be responsible for different cytochine production and may explain the absence of correlation.

Furthermore, it is known that puberty is associated with decreased insulin sensitivity and that pubertal development influences adiponectin levels and adipokine profiles (41-42); in our patients serum adiponectin and IL-10 levels showed no differences between sexes and pubertal stage but it is possible that the other adipokines implicated in the network of the Mets may influence the relationship between adiponectin and IL-10 and this might explain the lack of correlation.

Our data confirm that the mechanism implicated in the development of the MetS is complex and not yet fully elucidated.

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