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ORIGINAL ARTICLE

Interleukin-6, soluble interleukin-6 receptor/interleukin-6 complex and insulin resistance in obese children and adolescents

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Abstract
Background/aim To study the characteristics of interleukin 6 (IL6), soluble form of interleukin 6 receptor (sILR)/IL6 complex in obese children and adolescents and its relationship with insulin resistance (IR).
Subjects and methods 66 obese children and adolescents [34 boys, mean age 10.3 ± 2.9 years, *z*-score of body mass index (BMI) 4.76 ± 1.36] and 24 non-obese healthy sex- and age-matched controls. Fasting levels of glucose, insulin, IL6, sIL6, sgp130 were measured. IR was assessed by homeostasis model assessment of IR (HOMA-IR).
Results Obese subjects showed increased levels of insulin and IL-6 and higher HOMA-IR compared to controls (117.67 ± 50.9 vs. 62.42 ± 29.4 pmol/L, 2.73 ± 0.98 vs. 1.07 ± 0.41 pg/ml and 4.03 ± 2.16 vs. 1.83 ± 1.05 for insulin, IL-6 and HOMA-IR, respectively, $p < 0.01$ in all cases). sIL-6R levels were significantly lower in obese subjects (34.7 ± 14.2 vs. 55.6 ± 15.2 ng/ml in controls, $p = 0.005$), whereas sgp130 levels were not significantly different. In obese subjects, IL-6 directly correlated with *z*-score BMI ($r = 0.481$, $p = 0.009$) and with waist-to-height ratio ($r = 0.494$, $p = 0.007$), while sIL6-R was inversely related to HOMA-IR ($r = -0.522$, $p = 0.002$). Insulin resistant subjects showed higher levels of IL6 and lower levels of sIL6R (3.31 ± 0.72 vs. 2.25 ± 0.64 pg/ml, $p = 0.020$ and 25.3 ± 9.3 vs. 42.5 ± 10.4 ng/ml, $p = 0.013$, respectively).
Conclusions In obese children and adolescents, IR is associated with elevated levels of IL-6 and diminished values of sIL-6R.

Keywords Obesity · Insulin resistance · IL-6 · Transsignalling · Pediatrics

Abbreviations

BMI	Body mass index
HOMA-IR	Homeostasis model of assessment-insulin resistance
IR	Insulin resistance
IL-6	Interleukin 6
IL-6R	Interleukin 6 receptor
sIL-6R	Soluble form of interleukin 6 receptor

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Introduction

Childhood obesity has become an ever-increasing problem and is now considered a disease of epidemic proportions. There is substantial evidence that obesity in childhood lays the metabolic groundwork for adult type 2 diabetes, metabolic syndrome, and cardiovascular disease [1, 2].

Some experimental and clinical studies have demonstrated that interleukin-6 (IL-6) concentrations increase with weight gain and are associated with the development of type 2 diabetes and insulin resistance (IR) [3, 4].

IL-6 is an immunomodulatory cytokine belonging to the family of four helical cytokines and shows potent proinflammatory and endocrine actions. On target cells, this cytokine interacts with its receptor complex, which consists of the IL-6 receptor (IL-6R) and two molecules of gp130 and leads to the initiation of intracellular signaling. While gp130 is present on most cells of the body, IL-6R is only present on some cells, mainly hepatocytes and several leukocytes. Cells that only express gp130 and no IL-6R are refractory to the IL-6 signal [5]. These cells can be stimulated by a complex of IL-6 and the soluble form of IL-6R (sIL-6R) that is generated by proteolytic cleavage of IL-6R at the site adjacent to the transmembrane domain or by differential mRNA splicing. This protein renders cells that only express gp130 responsive towards the cytokine IL-6. The activity of the IL-6/sIL-6R complex is counteracted by the presence of a soluble form of gp130—sgp130—which tightly regulates the activity of the IL-6/sIL-6R complex. This pathway has been termed "transsignalling" [6]. It has been suggested that IL-6 exerts its anti-inflammatory actions via traditional signaling, whereas the proinflammatory effects, such as recruitment of mononuclear cells, are triggered through transsignalling [7].

Considering the prevalent influence of IL-6 in the pathogenesis of IR in obese subjects, we decided to evaluate all components of IL-6 biological systems, including sIL-6R and sgp130, in a group of obese children and adolescents. To the best of our knowledge, these parameters have been evaluated in few studies concerning adults [8].

Subjects and methods

Patients

A total of 125 consecutive obese patients (z-score of BMI >2) [9] belonging to an outpatient clinic for severe obesity of children and adolescents of Rummo Hospital (Benevento, Italy) were proposed to enter the study. Among 96 patients retained after considering selection criteria (see below), 66 accepted to participate to the study (34 boys and 32 girls of Caucasian Italian descent, mean age 10.3 ± 2.9 years, mean z-score BMI 4.76 ± 1.36, 48 prepubertal). All patients have early-onset obesity (i.e., onset before the physiologic time of "adiposity rebound", at the age of 6 years). 24 non-obese healthy sex- and age-matched were studied as controls. The latter were recruited among a group of children and adolescents investigated for idiopathic short stature in whom complete diagnostic work-up failed to reveal any organic disease.

Exclusion criteria were the presence of syndromes, anomalous growth pattern suggesting a secondary obesity, diabetes, the use of medication that alters glucose metabolism, familial dyslipidemia and/or incomplete medical data (i.e., absence of auxological data allowing the analysis of growth dynamic). A temporary exclusion criterion was the presence of an underlying illness at the time of the first examination. Subjects with a Tanner stage ≥2 were considered pubertal.

Auxological measurements

All anthropometric measurements were taken by the same well-trained investigator (GDF). Weight was measured to the nearest 0.1 kg with subjects standing barefoot in light clothing. Height was measured to the nearest 1 mm using a portable stadiometer, with the subject upright and the head in the Frankfurt plane. BMI was calculated as weight (kg)/height^2 (m^2) and expressed as a z-score [10]. Waist circumference (WC) was measured using a tape measure just above the uppermost lateral border of the right ilium at the end of a normal expiration and was recorded to the nearest millimeter, and the waist-to-height ratio (WTHR) (WC in cm/height in cm) was calculated.

The homeostasis model of assessment-insulin resistance (HOMA-IR) index was calculated by the following formula: glucose mmol/l × insulin mUI/l/22.5 [11]. IR was defined on the basis of a HOMA-IR higher than 95th percentile for sex and pubertal stage, according to Italian percentiles [12].

Laboratory procedures and assays

Enrolled subjects in complete well-being were directed to a centralized laboratory to provide blood samples after a 12 h overnight fast for the measurement of glucose, insulin, total cholesterol, triglycerides, high-density lipoprotein cholesterol, IL-6, sIL-6R, and sgp130. Serum samples were separated within 1 h of collection, stored in 1 ml aliquots, and kept frozen at −80 °C. All biochemical parameters were evaluated in duplicate and contextually for patients and controls.

Serum levels of IL-6, sIL-6R, and sgp130 were determined using commercially available ELISA kits

(Quantikine, R&D Systems, Minneapolis, USA) having sensitivity of 0.70 pg/ml, 7 and 0.08 ng/ml for IL-6, sIL-6R, and sgp130, respectively. The intra- and inter-assay CVs were <5 % for IL-6 and sgp130 serum levels, and <10 % for sIL-6R serum levels.

Statistical analysis

Results are expressed as mean ± standard deviation for continuous variables and as absolute (percent) values for discrete variables. The unpaired Student's *t* test was used for continuous variables to evaluate between-group differences for a given variable. For comparison of discrete variables, the χ^2 or Fisher's exact test was used. Pearson's correlation coefficient was used to determine relationships between different parameters. A *p* value lower than 0.01 was considered significant.

Ethical approval

The study was approved by the local ethical committee of Gaetano Rummo Hospital, Italy. Written parental informed consent and the assent of patients and controls were taken prior to their enrollment into the study.

Results

Clinical and biochemical characteristics of patients and controls are detailed in Table 1. As expected, obese subjects showed increased serum levels of insulin and IL-6 and higher HOMA-IR compared to healthy controls (117.67 ± 50.9 vs. 62.42 ± 29.4 pmol/L, 2.73 ± 0.98 vs. 1.07 ± 0.41 pg/mL and 4.03 ± 2.16 vs. 1.83 ± 1.05 for insulin, IL-6 and HOMA-IR, respectively, $p < 0.01$ in all cases). Moreover, serum levels of sIL-6R were significantly lower in obese patients compared to controls (34.7 ± 14.2 vs. 55.6 ± 15.2 ng/mL, $p = 0.005$), whereas sgp130 were not significantly different between the two study groups. In obese subjects, IL-6 levels directly correlated with *z*-score BMI ($r = 0.481$, $p = 0.009$; Fig. 1a) and with WTHR ($r = 0.494$, $p = 0.007$; Fig. 1b) while sIL6-R serum levels were inversely related to HOMA-IR index ($r = -0.522$, $p = 0.002$) (Fig. 2). The analysis after adjustment for age (in the former) and for age and *z*-score BMI (in the latter) confirmed these relationships, not observed in healthy controls.

When obese subjects were classified as IR ($n = 30$, 18 females, mean age 10.9 ± 3 years) and not IR ($n = 36$, 14 female, mean age 9.3 ± 1.6 years), the former showed higher levels of IL6 and lower levels of sIL6R (3.31 ± 0.72 vs. 2.25 ± 0.64 pg/mL, $p = 0.020$ and 25.3 ± 9.3 vs. 42.5 ± 10.4 ng/ml, $p = 0.013$ (Fig. 3).

Table 1 Anthropometric and metabolic characteristics of the study cohort

	Obese subjects	Healthy controls	*p*
Number	66	24	
Males:females	34:32	12:12	0.899
Mean age (years)	10.3 ± 2.9	9.8 ± 3.4	0.236
Prepubertal:pubertal	48:18	16:8	0.605
Z-score BMI (kg/m^2)	4.76 ± 1.36	1.39 ± 0.84	<0.001
Waist-to-height ratio	0.63 ± 0.03	0.52 ± 0.03	<0.001
SBP (mmHg)	100 ± 5.2	97.5 ± 10	0.474
(percentile)	40th	31st	0.474
DPB (mmHg)	65 ± 7.1	65.56 ± 6.83	0.798
(percentile)	60th	60th	0.798
Glucose (mmol/L)	4.86 ± 0.35	4.66 ± 0.39	0.089
Insulin (pmol/L)	117.67 ± 50.9	62.42 ± 29.4	0.001
HOMA-IR	4.03 ± 2.16	1.83 ± 1.05	0.002
Triglycerides (mmol/L)	0.87 ± 0.34	0.77 ± 0.37	0.648
Total cholesterol (mmol/L)	4.27 ± 0.72	4.08 ± 0.8	0.432
HDL-cholesterol (mmol/L)	1.29 ± 0.14	1.4 ± 0.16	0.517
LDL-cholesterol (mmol/L)	2.7 ± 0.9	2.31 ± 0.7	0.521
IL-6 (pg/mL)	2.73 ± 0.98	1.07 ± 0.41	0.009
sIL-6R (ng/mL)	34.7 ± 14.2	55.6 ± 15.2	0.005
s-gp130 (ng/mL)	302.8 ± 61.3	324.2 ± 65.1	0.142

Data are expressed as mean ± standard deviation. The subjects with a Tanner stage ≥2 were considered as pubertal

BMI body mass index, *HOMA* homeostasis model assessment, *IL-6* interleukin-6, *sIL-6R* soluble IL-6 receptor, *s-gp130* soluble gp130

p values were evaluated using the contingency table Chi-square tests and analysis of variance to test for between-group differences in non-parametric and parametric variables, respectively

P value lower than 0.05 was considered significant

Discussion

Our data demonstrate that a hallmark of IR in pediatric subjects resides in the association between increased levels of IL-6 and reduced levels of sIL-6R, and suggest that these abnormalities precede the onset of overt carbohydrate intolerance in insulin resistant subjects. This observation is not shared by other authors who found elevated levels of IL-6 but also of sIL6-R and gp130 in adult obese patients with metabolic syndrome [7]. One explanation for this discrepancy could be the duration of obesity, which is shorter by definition in our patients, and the raw number of adipocytes secreting IL-6 that is closely associated with obesity and IR [13].

Furthermore, previous reports in the literature evidenced that high levels of sgp130 were associated with hypertriglyceridemia, hypertension and elevated fasting glucose [8]; all findings absent in our cohort.

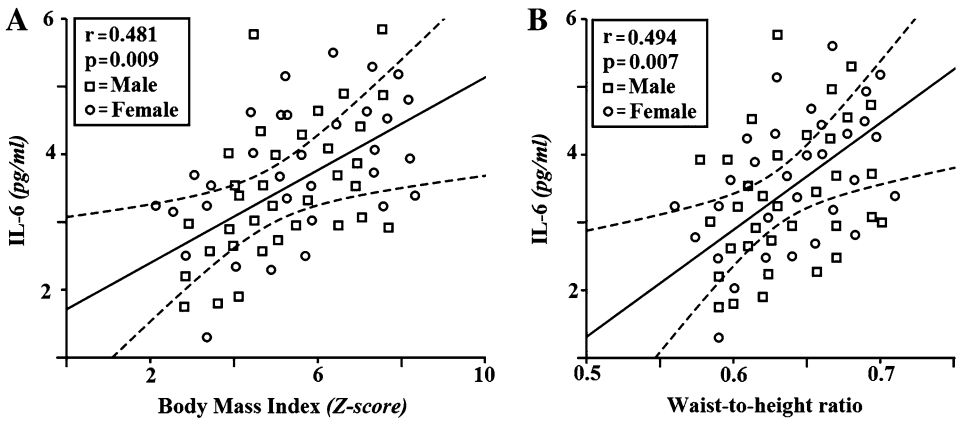

Fig. 1 **a** Direct correlation between IL-6 levels and *z*-score BMI in obese subjects ($r = 0.481$, $p = 0.009$). **b** Direct correlation between IL-6 levels and waist-to-height ratio ($r = 0.494$, $p = 0.007$). *Square*: males, *circles*: females

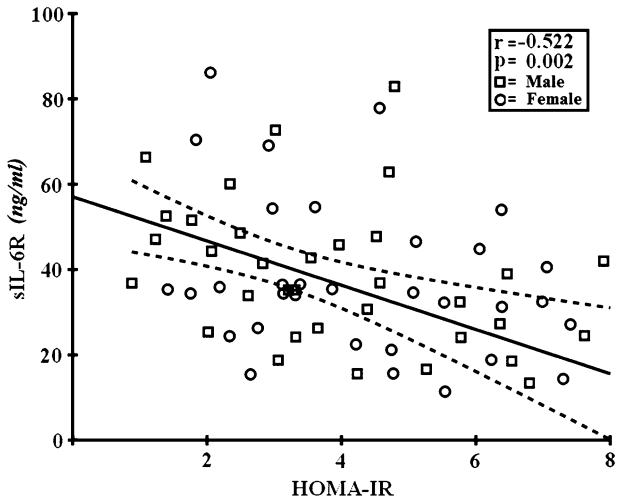

Fig. 2 Inverse correlation between sIL6-R serum levels and HOMA-IR index in obese subjects ($r = -0.522$, $p = 0.002$). *Square*: males, *circles*: females, *IR*: Insulin Resistant

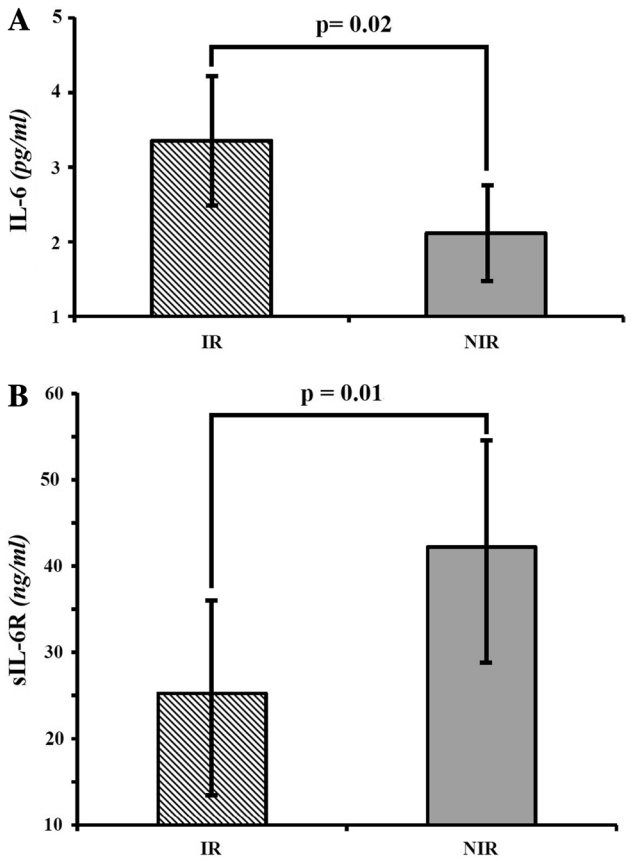

Fig. 3 IL6 and sILR levels in obese subjects with (i.e., insulin resistant, *IR*) and without (i.e., non-insulin resistant, *NIR*) insulin resistance

Adipose tissue IL-6 expression accounts for almost 30 % of systemic IL-6, and circulating IL-6 concentrations are positively correlated with obesity, impaired glucose tolerance, and IR [14].

Plasma IL-6 concentrations predict the development of type 2 diabetes [15], and peripheral administration of IL-6 induces hyperlipidemia, hyperglycemia, and IR in rodents and humans [16, 17].

Some studies have suggested that IL-6 could be involved in IR and its complications [18]. A mechanism of IL-6-induced IR in the liver has been proposed, which involves the activation of STAT3 (signal transducer and activator of transcription 3) and the subsequent induction of a suppressor of SOCS3 (cytokine signaling 3) [19]. Since hepatocytes express IL-6R, the biological actions of IL-6 in these cells were not mediated by sIL-6R. The lower circulating levels of sIL-6R in obese children could reduce the metabolic action of IL-6 in tissues without constitutive expression of IL-6R (i.e., adipose and skeletal muscle) and thus increase the relative availability of IL-6 for cells that constitutively express IL-6R, in particular hepatocytes. In these cells, IL-6 response through the membrane-bound IL-6R is not influenced by the reduced bioavailability of sIL-6R. The inverse relationship between sIL-6R levels and the HOMA-IR index observed in obese children confirms indirectly this

hypothesis. However, the reduced serum levels of sIL-6R observed in patients with higher levels of HOMA-IR could be considered a compensatory mechanism, attempting to determine a functional inhibition of this pathway, in order to reduce further IL-6-related systemic damage.

The strengths of the study are the strict selection criteria (permitting to exclude secondary and/or syndromic obesity) and the ethnical homogeneity of the studied population, avoiding confounding factors belonging to inter-ethnic variation in insulin sensitivity.

The major limit of our study is the choice of HOMA-IR and not of the euglycemic clamp to evaluate IR. In the literature, cut-off levels change from 2.5 for adults [20] to 4 for adolescents [21]. A study concerning adolescent patients suggested 3.16 [22]. We have chosen to define IR on the basis of Italian percentiles of HOMA-IR [12]. Furthermore, our data show a correlation with the degree of IR according to HOMA-IR index, independently from the cut-off point. Another limit of the study is the exclusion of a diabetic state on the basis of the medical history and fasting plasma glucose, without performing an oral glucose tolerance test and/or HbA1c dosage. It has to be argued that the young age of our cohort limits the risk of underestimating the diagnosis of diabetes in presence of normal fasting glucose levels.

Conclusions

Our data suggest that in pediatric and adolescent obese patients IR is associated with elevated levels of IL-6 and diminished values of sIL-6R. Over years, this perturbed pattern could precede the impairment of the entire system previously described in adults. Further experimental evidence for the suggested model is needed.

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Conflict of interest The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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