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Adiponectin profile and Irisin expression in Italian obese children: Association with insulin-resistance

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ABSTRACT

Adiponectin (Acrp30), its high molecular weight (HMW) oligomers, and Irisin are molecules involved in several metabolic processes. To investigate if these cytokines could represent new metabolic markers, we evaluated the expression of Acrp30 and Irisin in serum of obese children from South Italy affected by different degrees of insulin resistance (IR). The anthropometric and metabolic features were evaluated in 27 obese children versus 13 age-matched controls. The expression of Acrp30, its pattern and Irisin were investigated by ELISA, western blotting and fast protein liquid chromatography. The HOMA index was significantly higher in obese children versus controls, and metabolic syndrome was more prevalent in obese children with elevated IR versus those with normal HOMA (38% vs 16%). Total Acrp30 and HMW oligomers were significantly lower in obese than in control children, and the difference was more pronounced in children with HOMA >3.4. In control and obese children, total Acrp30 and HMW oligomers were inversely related to HOMA (r-0.38, *p* 0.02; r-0.35, *p* 0.03). Irisin was significantly higher in obese than in control children, and was inversely correlated with Acrp30 and HMW (r-0.32, *p* 0.04; r-0.39, *p* 0.01). The inverse correlation of Acpr30 and HMW oligomers with HOMA indicates that Acpr30 is directly involved in IR status. Moreover, the inverse correlation between Irisin and Acrp30 and, more significantly, between Irisin and HMW oligomers suggests that the two cytokines are closely connected. The use of Acrp30, HMW oligomers and Irisin as predictive factors of IR in obese children remains to be further elucidated.

1. Introduction

Obesity is a complex multifactorial disease resulting from the interaction of lifestyle, the environment and genetic variants. The prevalence of this disorder has increased worldwide and affects all age ranges including the pediatric population [1]. Overweight and obesity in children is a major public health problem because it often leads to adult obesity, which is associated with an enhanced risk of type 2 diabetes (T2DM), metabolic syndrome (MS), cardiovascular diseases (CVD), and different cancers [2–4]. In addition, high insulin resistance (IR) is a harbinger of T2DM in obese children.

Adipose tissue and skeletal muscle are endocrine organs that secrete adipokine and myokine hormones, respectively and are thus critical for the regulation of energy metabolism and inflammation. Dysregulation of adipokine and myokine secretion plays a pivotal role in the development and progression of both obesity and IR in adults as well as in children [5]. Being involved in the regulation of energy expenditure, insulin sensitivity and inflammation, adipokines are promising molecular targets for the treatment of obesity and its related diseases [6]. Adiponectin (Acrp30) is widely recognized for its anti-diabetic, anti-inflammatory, and cardio-protective effects [7]. Acrp30 is a protein hormone of 244 amino acids that circulates at high concentrations (5–30 μg/mL), and accounts for 0.01% of total serum proteins. It is synthesized as a monomer of 28–30 kDa that assembles in oligomers of various molecular weights: low molecular weight (LMW), medium molecular weight (MMW), and high molecular weight (HMW). The

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latter are the most active oligomers in terms of insulin sensitivity modulation and anti-inflammatory activities [6]. Indeed, total Acrp30 and HMW levels are reduced in adult obesity and in its associated metabolic complications [8,9]. In obese children, Acrp30 has been correlated to such metabolic factors as IR [10,11]. The myokine Irisin is a protein hormone that plays a role, together with adipokines, in the maintenance of energy balance [12]. In fact, Irisin increases energy expenditure and glucose tolerance, which implicates it in the pathogenesis of various complications of obesity including IR and T2DM. Accordingly, high circulating concentrations of Irisin have been reported in patients affected by IR [12].

Although Acrp30 and Irisin have been closely associated to obesity, IR and diabetes [6,7,12], they have not yet been simultaneously analyzed in either children or adults affected by obesity, and/or IR. In the attempt to identify early markers of IR in obese children, we analyzed serum levels of total Acrp30, its oligomeric distribution and Irisin levels in obese children from South Italy affected or not by IR with those of sex- and age-matched lean children. We also investigated correlations between total Acrp30, and/or Acrp30 HMW oligomers with Irisin. Finally, we investigated whether the two cytokines were correlated with anthropometric/metabolic parameters, and/or IR and/or metabolic syndrome.

2. Methods

2.1. Subjects and anthropometric and biochemical measurements

We enrolled 27 obese children from South Italy (age ranging from 4 to 13 years) and 13 sex- and age-matched lean controls attending the Department of Woman, Child and General and Specialized Surgery of the Second University of Naples (Italy). All procedures were in accordance with the Helsinki Declaration of Principles and approved by the local ethics committee. Written informed consent was obtained from all parents or guardians. Physical examination included weight and height evaluation and waist/height ratio and Z-scores to calculate the body mass index (BMI) [13]. We assessed pubertal stage according to Tanner's criteria [13]. Systolic and diastolic blood pressure was measured and standard deviation scores calculated (SBP-SDS, and DBP-SDS). A blood sample was drawn at 8 a.m. and total cholesterol, HDL, LDL, triglyceride, glucose, aspartate transaminase (AST), and alanine transaminase (ALT) levels were measured. Obesity, MS and IR were defined as previously described [14]. Patients were deemed to have increased IR when the HOMA index exceeded 3.4 [15]. Total Acrp30 was measured by ELISA using house-produced polyclonal antibodies [16], and with a commercial kit (Millipore, MA, USA). HMW oligomers in serum were detected using a commercial kit (Millipore, Billerica, MA, USA). Serum Irisin concentrations were measured also using a commercial ELISA kit (Phoenix Pharmaceuticals, Belmont, CA, USA). The lowest detectable concentration of Irisin was 2.06 ng/ml and highest was 36.12 ng/mL. Each serum sample was tested three times in triplicate.

2.2. Western blotting analysis

Ten micrograms of serum proteins were treated as previously described (8, 9). All samples were tested twice in duplicate. Incubation with Acrp30 and Irisin antibodies (Novus Biologicals, Littleton, CO, USA) was performed according to the manufacturer's instructions. Blots were developed by enhanced chemiluminescence (ECL) with Kodak BioMax Light film, (GE Healthcare Bio-Sciences Pittsburgh, PA, USA), digitalized with a scanner (1200 dpi) and analyzed by densitometry with ImageJ Software (http://rsbweb.nih.gov.ij/).

2.3. Gel filtration analysis

The Acrp30 oligomeric pattern was analyzed with a Superdex 200 10/300 GL column connected to a fast protein liquid chromatography system (GE Healthcare Bio-Sciences Pittsburgh, PA, USA) as reported elsewhere [8,9]. In detail, 1,875 mg of total proteins were fractionated at 0.5 ml/min using PBS elution buffer. Fractions (500 μl) were collected and Acrp30 was tested using ELISA (100 μl) and western blotting (20 μl). The column was calibrated using ferritin (440 kDa), aldolase (158 kDa), and ovalbumin (44 kDa) (GE Healthcare Bio-Sciences Pittsburgh, PA, USA). This analysis was performed on 4 controls, 4 obese subjects with HOMA <3.4 and 4 obese subjects with HOMA >3.4.

2.4. Statistical analysis

Data were analyzed using the SPSS (v 10.0) Software Package (SPSS, Inc., Chicago, IL, USA). The significances of anthropometrical and biochemical parameter differences were determined using the *t*-test for normally distributed variables and the Mann–Whitney *U* test for non parametric variables. The chi square test was used to compare categorical factors. A multiple logistic regression analysis and general linear model were performed to correct the significant *p* values obtained by the univariate analysis. The correlations between Acrp30 levels or HMW oligomers and both HOMA and Irisin levels were determined using the Spearman's test. Statistical significance was established at $p < 0.05$.

3. Results

3.1. Anthropometrical and biochemical features of children

Anthropometrical and biochemical features of the children enrolled in the study are reported in Table 1. Obese children had significantly higher BMI Z-scores, waist-to-height ratios, and SBP-SDS, DBP-SDS, Chol-LDL, triglycerides, glucose and insulin levels than controls ($p < 0.01$); the HOMA values indicated that the obese children had significantly high IR ($p < 0.02$). In addition, both total Acrp30 levels and HMW isomer levels were significantly lower in obese children than in controls (total Acrp30: 14.6 ± 4 μg/mL *vs* 18.9 ± 7.8 μg/mL; *p* 0.02; HMW isomers: 8.3 ± 4 *vs* 6 ± 2.5 ± 4.1 μg/mL, *p* 0.03) (Fig. 1A). In obese children but not in controls, there was an inverse relationship between total Acrp30

Table 1
Comparison of anthropometric and biochemical features between controls and obese children. Data are expressed as mean ± S.D.

	Controls	Obeses	p-value
Number (boys)	13 (4)	27 (19)	0.8
Age (years)	8 ± 2.4	9.7 ± 2.7	0.8
BMI Z-score	−0.46 ± 1.2	2.7 ± 0.5	**0.00001**
Waist/height ratio	0.45 ± 0.02	0.62 ± 0.04	**0.01**
SBP SDS (mmHg)	−0.1 ± 0.9	0.7 ± 1.3	**0.01**
DBP SDS (mmHg)	−0.5 ± 0.5	0.8 ± 0.9	**0.01**
Total cholesterol (mg/dL)	150 ± 17	152 ± 27	0.8
LDL (mg/dL)	73 ± 12	83 ± 23	**0.05**
HDL (mg/dL)	60 ± 4	47 ± 9.5	**0.01**
Triglycerides (mg/dL)	55 ± 3.5	104 ± 48	**0.007**
ALT (U/L)	18 ± 13	29 ± 13	0.3
AST (U/L)	23 ± 4.5	23 ± 45.9	0.9
Glucose (mg/dL)	73 ± 7	80 ± 6	**0.03**
Insulin (μU/L)	6 ± 4	15 ± 7	**0.01**
HOMA	1.62 ± 0.3	3 ± 1.4	**0.02**
Metabolic syndrome (%)	0	30%	**0.01**

SBP SDS, systolic pressure; DBP SDS, diastolic pressure; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Acrp30, adiponectin; HMW, high molecular weight oligomers.

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