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C-type natriuretic peptide plasma levels are reduced in obese adolescents

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ABSTRACT

The high prevalence of obesity in children may increase the magnitude of lifetime risk of cardiovascular disease (CD). At present, explicit data for recommending biomarkers as routine pre-clinical markers of CD in children are lacking. C-type natriuretic peptide (CNP) is assuming increasing importance in CD; in adults with heart failure, its plasma levels are related to clinical and functional disease severity. We have previously reported five different reference intervals for blood CNP as a function of age in healthy children; however, data on plasma CNP levels in obese children are still lacking. Aim of this study was to assess CNP levels in obese adolescents and verify whether they differ from healthy subjects. Plasma CNP was measured in 29 obese adolescents (age: 11.8 ± 0.4 years; BMI: 29.8 ± 0.82) by radioimmunoassay and compared with the reference values of healthy subjects. BNP was also measured. Both plasma CNP and BNP levels were significantly lower in the obese adolescents compared to the appropriate reference values (CNP: 3.4 ± 0.2 vs 13.6 ± 2.3 pg/ml, $p < 0.0001$; BNP: 18.8 ± 2.6 vs 36.9 ± 5.5 pg/ml, $p = 0.003$). There was no significant difference between CNP values in males and females. As reported in adults, we observed lower plasma CNP and BNP levels in obese children, suggesting a defective natriuretic peptide system in these patients. An altered regulation of production, clearance and function of natriuretic peptides, already operating in obese adolescents, may possibly contribute to the future development of CD. Thus, the availability of drugs promoting the action of natriuretic peptides may represent an attractive therapeutic option to prevent CD.

1. Introduction

Obesity is the result of a chronic caloric imbalance, when more calories are assumed than consumed each day. Most obese adults were obese as adolescents and most adolescents were overweight and/or obese as children [40]; in fact, the origins of obesity are being traced to early childhood development. Childhood obesity is a worldwide health problem and its prevalence is increasing steadily and dramatically worldwide [44]. Obese children have a much greater likelihood than their normal-weight counterparts of acquiring dyslipidemia, hypertension and impaired glucose metabolism, with an increased risk of developing cardiovascular and metabolic diseases as adults [33]. Childhood obesity is also a major risk for early onset of endothelial dysfunction and atherosclerosis [30,48]. At present, explicit data for recommending biomarkers as routine pre-clinical indices of cardiovascular disease (CD) in children are lacking. C-type natriuretic peptide (CNP), a member of natriuretic cardiac peptides, is assuming increasing importance in cardiovascular disease. In adults a relationship between plasma CNP levels and clinical and functional disease severity was observed, especially in heart failure [10–15,20,22,35,46,49]. Recently, five different reference intervals for blood CNP, as a function of age, were reported in healthy children [16]. In addition, it has been shown that natriuretic peptides play a central role in the regulation of body weight and energy metabolism [32]. Interestingly, it has been shown that ANP and BNP are significantly lower in overweight and obese adult subjects than in the lean ones [8,18,21], probably contributing to insulin resistance and hypertension. Data on natriuretic peptides in children of pediatric age are scarce and limited to CNP in infancy [36–38], while data on plasma CNP levels in obese children are still lacking.

Aims of the present study were to assess how plasma CNP levels behave in obese adolescents compared to normal weight subjects, and whether they show the same pattern observed for BNP and ANP in adult obese patients. To better describe the

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Table 1
Clinical characteristics of the studied subjects.

Morphometric features (cases, $n = 29$)	
Sex	17 ♂–12 ♀
Age (years)	11.77 ± 0.4
Pubertal stage	1: $n = 7$ 2: $n = 12$ 3: $n = 5$ 4: $n = 4$ 5: $n = 1$
Height (cm)	154.43 ± 2.2
Weight (kg)	71.75 ± 2.9
BMI	29.8 ± 0.8
Obese/overweight	Obese, $n = 25$ Overweight, $n = 4$
Fat mass, FM (%)	37.1 ± 1.2
Fat mass, FM (percentile)	>95, $n = 8$ >98, $n = 21$
Systolic pressure, SBP (mmHg)	109 ± 2
Systolic pressure, SBP (percentile)	55.25 ± 4.9
Diastolic pressure, DBP (mmHg)	65 ± 1
Diastolic pressure, DBP (percentile)	52.7 ± 3.7

Table 2
Laboratory findings of the studied subjects.

Biochemical parameters	Case ($n = 29$)	Reference cut-off
Glycemia (mg/dl)	72.5 ± 1.9	<100 mg/dl
Insulin (uU/ml)	21.3 ± 2.2	<15 uUI/ml
HOMA-IR	4.0 ± 0.4	Males: 1.37 ± 0.7 Females: 1.65 ± 1.1
HOMA-IR (*Z*-score)	2.9 ± 0.5	–
Cholesterol (mg/dl)	172 ± 7	<180 mg/dl (<75th percentile) acceptable >210 mg/dl (>95th percentile) elevated
HDL (mg/dl)	45 ± 2	>37 mg/dl (>5th percentile)
LDL (mg/dl)	107 ± 5	<110 mg/dl (<75th percentile) acceptable >135 mg/dl (>95th percentile) elevated
Triglycerides (mg/dl)	97 ± 10	<110 mg/dl (<75th percentile) acceptable >170 mg/dl in females (>95th percentile) elevated >150 mg/dl in males (>95th percentile) elevated

neuroendocrine profile, BNP was also measured in the same samples.

2. Methods

2.1. Subjects and plasma collection

Twenty-nine overweight/obese adolescents (17 M, 12 F, age 11.8 ± 0.4 years), without cardiac dysfunction, referred as outpatients to the Unit of Pediatric Endocrinology and Diabetes, Department of Pediatrics, University of Pisa, Italy, were enrolled in the study.

Table 1 summarizes the clinical details of each participant in the study. BMI, calculated using the formula weight (kg)/height $(m)^2$, was 29.8 ± 0.82, corresponding to 2.9 ± 0.7 *Z*-scores [6]. According to the definition of the international Task Force on Obesity in childhood and using population reference data specific for age and sex for BMI [7], 25/29 were obese while the remaining 4 were overweight. Total body fat, measured using the Tanita BC-418 Segmental Body Composition Analyser (Tanita Corporation, Tokyo, Japan) showed that our patients had FM% in the range of obesity [27].

Blood pressure was measured by the same investigator using a standard validated protocol. In two subjects systolic blood pressure was above the 95th percentile for height, age, and sex-specific reference values [45] indicating a condition of systolic hypertension.

Table 2 summarizes the laboratory characteristics of each participant in the study. Fasting plasma glucose (FPG) was normal in all. Fifteen subjects showed HOMA-IR (HOmeostasis Model Assessment of Insulin Resistance) values > 2.0 *Z*-scores, suggesting that they had a reduced insulin sensitivity. This index was calculated according the formula: fasting plasma insulin in uU/ml × FPG in mmol/L/22.5 [26]. The results were compared with reference values specific for Italian children and adolescents, obtained using the same formula [9].

Regarding blood lipids, according to reference values specific for age and sex [43], four patients had levels of total cholesterol above the 95th percentile; four had LDL cholesterol above the 95th percentile, while tryglycerides were increased in six. Blood levels of HDL cholesterol were lower than 5th percentile in four patients [43].

Blood samples were collected in all the subjects by venipuncture, in the morning after an overnight fasting. In order to minimize degradation, blood samples for CNP were collected in ice-chilled disposable polypropylene tubes containing EDTA (1 mg/ml) and aprotinin (500 KIU/ml) to prevent proteolysis. Samples were rapidly separated by centrifugation for 15 min at 4 °C, and plasma stored frozen at −80 °C in 1-ml aliquots in polypropylene tubes until assay, performed within 1 month. Blood samples for blood glucose and lipids assays were collected in lithium-heparin containing vials, while insulin in EDTA containing vials.

The study was conducted in accordance with the guidelines proposed in the Helsinki Declaration and approved by the local ethics committee. Informed consent was obtained from the parents of each subject.

2.2. Biochemical parameters assays

A Cobas Integra 400 analyser (Roche, Italy) and the appropriate commercial kits were used to measure blood glucose (Cobas Integra 400 Glucose HK; enzymatic reference method with hexokinase), total cholesterol (Cobas Integra 400 Cholesterol; enzymatic, colorimetric method with cholesterol esterase, cholesterol oxidase, and 4-aminoantipyrine), HDL and LDL cholesterol fractions (Cobas Integra 400 HDL-Cholesterol and LDL-Cholesterol plus 2nd generation; homogeneous enzymatic colorimetric assays) and tryglicerides (Cobas Integra 400 Tryglicerides; enzymatic, colorimetric method with glycerol phosphate oxidase and 4-aminophenazone).

Circulating insulin levels were measured by a commercial immunoassay kit (Access® Ultrasensitive Insulin, Beckman Coulter Inc., Fullerton, CA, USA), with a sensitivity of 0.03 μIU/mL and a precision of <10% CV.

2.3. CNP, BNP assay

CNP was directly measured in plasma by a specific radioimmunoassay (RIA) (Phoenix Pharmaceuticals, Belmont, CA, USA). Each sample was determined in duplicate and the assay was carried out on ice. A control sample, prepared by using known amounts of CNP added to the assay buffer and stored in aliquots to −80 °C, was assayed in each run for quality control.

As reported in a previous study of ours [16], the working range was derived by the mean dose–response curve for CNP assay and the mean imprecision profile, calculated by a previously described computer program [34]; at the low range (CNP concentration: 5–80 pg/tube) the CV% resulted lower than 20%. CNP in vitro-stability was evaluated measuring a plasma pool in different experiments performed during 12 months and the between-assay

Table 3
Reference intervals for CNP and BNP plasma levels in childhood (modified by Ref. [16]).

Age reference intervals	CNP (pg/ml)	BNP (pg/ml)
0–3 days	11.6 ± 2.1	396.2 ± 100.3
4–30 days	16.4 ± 3.7	99.6 ± 36.3
1–12 months	15.4 ± 2.7	29.2 ± 3.92
1–12 years	13.6 ± 2.3	37.2 ± 5.7

variability resulted <30% ($n = 7$). Within-assay variability was evaluated using pools with different CNP concentration and both resulted < 20%: 7.05 ± 0.2 pg/tube ($n = 9$ duplicate assays, CV = 11%) and 12.4 ± 1.1 pg/tube ($n = 5$ duplicate assays, CV = 18%) pg/tube. Analytical sensitivity resulted 0.77 ± 0.05 pg/tube.

Plasma BNP was measured using the fully automated Access platform (Triage BNP reagents, Access Immunoassay Systems, REF 98200; Beckman Coulter Inc., Fullerton, CA, USA). The analytical characteristics and performance of the Access immunoassay method used in this study for measurement of BNP were previously evaluated [5] as well as the reference ranges in childhood [39].

Plasma CNP and BNP results were compared with appropriate reference values specific for age and sex [16]. In Table 3 the reference intervals for CNP and BNP plasma levels in childhood, obtained in a previous study of ours [16], were reported.

2.4. Statistical analysis

All sample values and other data for quality control of the RIA system were calculated using a previously described computer program [34]; the interpolation of the dose–response curves was computed using a four-parameter logistic function [34]. Because plasma CNP values are not normally distributed, natural logarithmic transformation of data was used for statistical analysis when needed. Differences of CNP levels between two independent groups were assessed by unpaired *t*-test. Relations between variables were assessed by linear regression. Results are expressed as mean ± SEM and a *p*-value < 0.05 was considered significant.

3. Results

Plasma CNP resulted significantly lower ($p < 0.0001$) in the obese subjects in comparison with the appropriate reference values (Fig. 1a) [16]. BNP plasma levels were also compared with the appropriate reference values previously determined in healthy children (37.2 ± 5.7 pg/ml) [16] and showed significantly lower levels ($p = 0.003$) in obese children with respect to controls, mimicking the trend already observed in obese adults (Fig. 1b). When the results were analyzed as a function of gender, no significant difference for CNP values was found between males and females. A significant positive correlation between BNP and glycemia ($r = 0.33$; $p < 0.05$) was observed.

Considering the biochemical parameters analyzed in obese children (glycemia, insulin, cholesterol, HDL, LDL and triglycerides) the only significant correlations resulted between CNP and HDL ($r = 0.37$, $p = 0.048$) as well as between BNP and HDL ($r = 0.4$, $p = 0.029$) and triglycerides ($r = 0.5$, $p = 0.005$) confirming their role in lipolysis and adipogenesis [3,32].

No significant correlation were observed between CNP or BNP and the morphometric features.

4. Discussion

This study reports for the first time the behavior of CNP plasma levels in obese adolescents, showing that overweight and obese children have significantly lower levels than normal-weight subjects. BNP plasma levels measured in the same subjects also resulted lower, confirming reports from previous investigators in obese adults [21,28,29,32,47] and suggesting a defective natriuretic peptide system in these patients.

There are several mechanisms that could be potentially responsible for the inverse association between natriuretic peptide plasma levels and body mass index [2,24]. One of these may be an increased expression of the natriuretic peptide clearance receptors (NPR-C) on adipocyte cells and/or impaired synthesis/release of natriuretic peptides from myocytes found in obese subjects [1]. Another mechanism for the reduced secretion of natriuretic peptides might be an impaired myocardial hormone release [23] or synthesis [31] as a consequence of overweight/obesity. Recently, we observed lower levels of BNP, ANP and CNP mRNA expression in cardiac tissue of obese rats in comparison with their normal-weight counterparts, suggesting that obesity may be associated with a decreased synthesis of these peptides by cardiac cells [3]. Thus, we hypothesize that a similar obesity-induced mechanism might also be operating in obese humans, including children and adolescents.

Thus, in our overweight/obese adolescents a decreased synthesis and increased clearance may work synergistically to lower the circulating levels of natriuretic peptides.

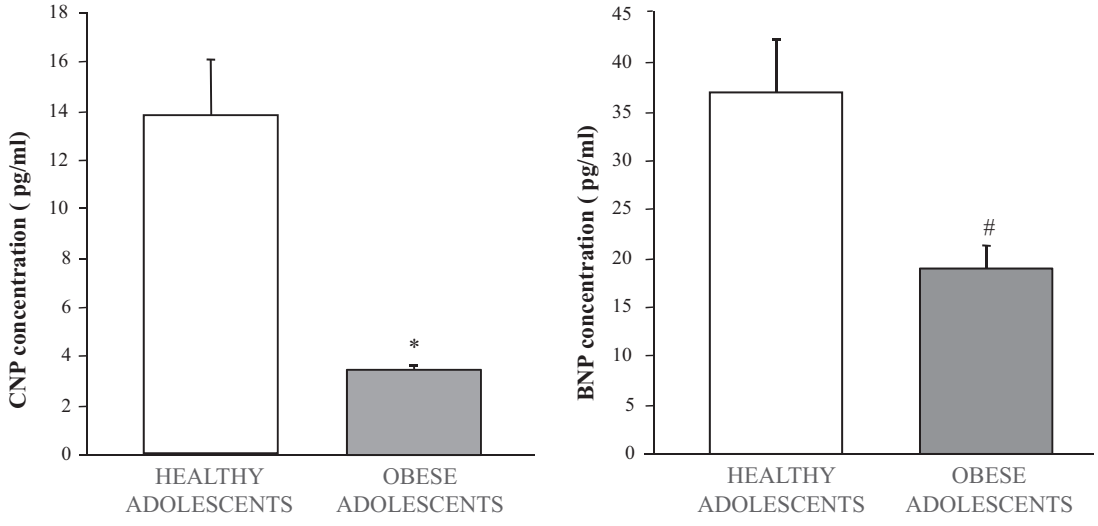

Fig. 1. CNP and BNP plasma levels in healthy and obese adolescent. White box: healthy subjects ($n = 32$), gray box: obese adolescents ($n = 29$). *$p < 0.0001$, #$p = 0.003$.

Alternatively, it has been hypothesized that the decreased levels of circulating natriuretic peptide are not the consequence of overweight/obesity, but rather a causative factor in the genotype or phenotype leading to development of obesity [21]. In fact, both ANP and BNP are now recognized factors involved in fat metabolism as stimulators of lipolysis in adipose tissue [19].

Data on plasma natriuretic peptide levels in obese children and adolescents are scarce, limited to pro-BNP and not univocal. To our knowledge there are only two studies in obese children/adolescents, showing contrasting results. One study reported higher levels of pro-BNP in obese vs normal-weight controls [41], while the other study did not find any difference between obese and non-obese subjects [42]. Both studies suggest that circulating pro-BNP in obese children/adolescents behaves differently than in obese adults, who have reduced levels of the peptide [21,28,29,32,47]. While our results agree with those found in obese adults, they do not agree with data reported in children/adolescents [41,42]. We do not have a clear-cut explanation for the different results in circulating BNP levels found among our own and others' studies. One possibility might be the different obese populations examined, which were younger than ours, spanning from about age 5 to 14 years [41] and from age 5 to 17 years [42], and the different assay method used. It is known that BNP and NT-proBNP results depend on the assay method used and on the age and gender of the population studied [4].

In recent years, the childhood obesity epidemic has begun to compromise the health of the pediatric population by promoting premature development of atherosclerosis and metabolic syndrome, both of which significantly increase the risk of cardiovascular disease early in life. Given the involvement of natriuretic peptides in these conditions [17,25], it becomes extremely important to have age-specific reference intervals for this peptide, as reported in some recently published studies on natriuretic peptides [4,16,27].

A limitation of this study is the lack of control subjects. However, we used our own reference data, specific for the BNP and CNP assay methods employed in this study. It is also interesting to note that both BNP and CNP had the same pattern in our overweight/obese subjects, supporting that an altered regulation of natriuretic peptides may be working at this age, as previously observed in adults. These data suggest that CNP determination, along with that of the other natriuretic peptides, may be considered a complementary tool for the characterization of these subjects. In this regard, the availability of drugs promoting the actions of natriuretic peptides may be an attractive therapeutic option for preventing cardiovascular disease.

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