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C-type natriuretic peptide is closely associated to obesity in Caucasian adolescents

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ABSTRACT

CNP is a natural regulator of adipogenesis playing a role in the development of obesity in childhood. Aim of the study was to evaluate CNP plasma levels in normal-weight (N), overweight (OW) and obese adolescents (O). Eighty two subjects (age:12.8 ± 2.4, years) without cardiac dysfunction were enrolled and CNP plasma levels were measured by RIA. NT-proBNP, MR-proANP, AGEs, reactive hyperemia index (RHI) and standard clinical chemistry parameters were also measured. O and OW adolescents had higher values of BMI and fat mass than N. CNP levels were significantly lower in OW:4.79[3.29–21.15] and O:3.81[1.55–13.4] than in N:13.21[7.6–37.8]; $p < 0.0001$ N vs O, $p = 0.0003$ N vs OW). LogCNP values correlated significantly and inversely with BMI z-score, FM%, TF% and circulating levels of CRP, insulin, total cholesterol, LDL, and triglycerides, in addition to an inverse relationship with skin AGEs and a direct correlation with RHI. LogCNP was also inversely associated with LogNT-proBNP and LogMR-proANP values. Using ROC analysis the risk of obesity resulted significantly ($p \ll 0.0001$) associated with CNP values (AUC = 0.9724). These results suggest that CNP may play a more important role than BNP and ANP related peptides, as risk marker of obesity, in addition to its involvement in adipogenesis and endothelial dysfunction.

1. Introduction

Although the role of Atrial Natriuretic peptide (ANP) and B-type Natriuretic Peptide (BNP) on lipolysis/lipogenesis pathways was studied in detail [1–4], less is known on the action of third member of the Natriuretic Peptide family, the C-type Natriuretic Peptide (CNP) [5–7]. There are only few experimental studies evaluating CNP and NPR-B expression in rats [6,7]. One more study suggested that CNP may play a physiological autocrine/paracrine role, as early regulator of adipogenesis, by binding to the specific receptor NPR-B and activating cGMP-dependent pathways [5]. Data in humans are lacking, especially during paediatric age. To our knowledge, there is only a preliminary study reporting CNP plasma levels in children and adolescents with obesity [8].

More is known on vascular effects of CNP. Several data indicate that CNP plays a pivotal role in the regulation of endothelial function [9–12]. Since CNP is widely expressed throughout all vasculature wall (especially in endothelium), where it induces vasorelaxation by hyperpolarization [9,10], it may be that changes in the peptide levels might contribute in developing vascular complication of obesity already during childhood [9–12].

However, there are some pathophysiological and methodological considerations to take into account when plasma levels of CNP are measured and compared to those of ANP and BNP, especially in children [13–16]. While ANP and BNP exert their physiological actions on all tissues of human body as circulating hormones, binding to the specific NPR-A [3,4,13,14], CNP is thought to have mainly a paracrine action, especially in organs rich in endothelial cells [13]. Thus, CNP and peptide cardiac hormones can share different physiological actions, even in the same tissue, due to both different paracrine or hormonal actions and receptor binding. NT-proBNP has been investigated in children with obesity giving contrasting results [15–17]. As regards MR-proANP, the mid

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regional part of proANP, it emerged as a promising biomarker of cardiac abnormalities, but data on its clinical utility in the general population, especially in paediatric age, are still limited [18]. From a methodological point of view, plasma concentration of BNP and ANP (and their related peptides, such as NT-proBNP and MR-proANP) are routinely measured in clinical laboratories by means of fully automated, highly sensitive, non-competitive immunoassay methods, which are able to accurately measure peptide hormone concentration from neonatal period up to senescence [15,16]. On the contrary, plasma levels of CNP and its related peptides are measured by competitive manual immunoassays, like radioimmunoassay (RIA) or enzyme-immuno-assay (EIA) methods, which are usually less specific and sensitive than automated non-competitive immunoassay methods. For these reasons, CNP RIA and EIA methods are not commercially available for clinical laboratory routine, but used only in research laboratories. Furthermore, it is well known that there are some difficulties to collect plasma samples for research studies in paediatric age due to methodological as well as ethical considerations [16,19,20]. These difficulties in measuring together with pathophysiological interpretations of plasma CNP explain the limited number of studies on CNP compared with those on BNP during paediatric age.

The main aim of this study was to test the hypothesis whether circulating CNP and/or cardiac natriuretic peptide levels were significantly related to both obesity and some risk markers of atherosclerosis in adolescent subjects with obesity. Therefore, we evaluated plasma levels of CNP, NT-proBNP and MR-proANP, as well as growth and body composition parameters, some cardio-metabolic risk factors, and the amount of skin advanced glycated endproducts (AGEs) in normal weight, overweight and young adolescents with obesity [21,22].

2. Materials and methods

2.1. Subjects and plasma collection

Eighty two subjects (age:12.8 ± 2.4, years) without cardiac dysfunction, referred as outpatients to the Unit of Pediatric Endocrinology and Diabetes, Department of Clinical and Experimental Medicine, University of Pisa, Italy, were enrolled in the study and divided into normal weight (N), overweight (OW) and adolescents with obesity (O) (see Table 1a), 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 [23]. Normal weight adolescents were healthy subjects, who repeated blood examination after an intervening disease. They were not assuming drugs from at least one week before blood sampling and showed normal blood results, in particular indices of inflammation were in the normal range. BMI was calculated using the formula weight [(kg)/height (m)^2] [24]. We used the same National reference data [24] to calculate BMI z-score and Height z-score. Table 1a summarizes the clinical details of each group. Total body fat (%) and trunk fat (TF) were measured using the Tanita BC-418 Segmental Body Composition Analyser (Tanita Corporation, Tokyo, Japan) [25]. Blood pressure was measured by trained investigators according to a standardized protocol [26]. Briefly, blood pressure was taken on the left arm with the subject sitting, using an aneroid sphygmomanometer; the cuff had bladder long enough to encircle at least one-half of the upper arm without overlapping and widths that covered at least two-thirds of the upper arm. The average of three blood pressure values was used for analysis.

Blood samples were collected in all the subjects by venipuncture, in the morning after an overnight fasting. Blood samples to assay natriuretic peptides were collected in ice-chilled disposable polypropylene tubes containing EDTA (1 mg/mL) and aprotinin (500 KIU/mL) to prevent proteolysis and minimize peptide degradation [15]. Blood samples to evaluate blood glucose and lipids were collected in lithium-heparin containing vials, while those for insulin assay were gathered in EDTA containing vials. After collection, samples were quickly separated by centrifugation for 15 min at 4 °C, and plasma was stored frozen at −80 °C, in 1-mL aliquots, in polypropylene tubes until assay that was performed within 1 month. HOMA-IR (Homeostasis Model Assessment of Insulin Resistance) index was calculated according the formula: fasting plasma insulin in uU/mL x FPG in mmol/L/22.5 [27,28].

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. Advanced glycated end- products (AGEs)–dependent skin autofluorescence measurements

AGE-dependent skin autofluorescence (AF) was measured by the AGE Reader apparatus (DiagnOptics, Groningen, the Netherlands) [29]. In accordance with the manufacturer's instructions, measurements were taken at three different sites on the inner side of the arm, with the subject in a seated position. AF was expressed in arbitrary units.

2.3. Biochemical parameter assays

Blood glucose, total cholesterol, HDL and LDL cholesterol fractions and triglycerides were measured by a Cobas Integra 400 analyzer (Roche, Italy) using appropriate commercial kits (Cobas Integra 400 Glucose HK; enzymatic reference method with hexokinase, Cobas Integra 400 Cholesterol; enzymatic, colorimetric method with cholesterol esterase, cholesterol oxidase, and 4-aminoantipyrine, Cobas Integra 400 HDL-Cholesterol and LDL-Cholesterol plus 2nd generation; homogeneous enzymatic colorimetric assays, Cobas Integra 400 Triglycerides; enzymatic, colorimetric method with glycerol phosphate oxidase and 4-aminophenazone).

HbA1c was assessed by HPLC (G7 HPLC Analyzer, Tosoh Bioscience Inc., South San Francisco, USA; reference range 23–43 mmol/mol).

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

Serum CRP levels were assayed by the high-sensitive CRP immunoturbidimetric method (Kamiya Biomedical Co., Seattle, WA, USA) with a sensitivity of 0.005 mg/dL and intra- and interassay CVs of 1.24 and 4.2%, respectively.

Table 1a
Clinical characteristics of adolescents at normal weight, overweight and with obesity.

	Normal weight subjects N = 27	Overweight subjects OW = 10	Subjects with obesity O = 45
Age	12.8 ± 1.4	12.6 ± 1.6	12.4 ± 2.1
Male:female	13:14	5:5	26:19
Pubertal stage (Tanner score)	2.7 ± 1*	3.5 ± 1.2	3.7 ± 1.3*
Height, cm	156 ± 11	161 ± 8.2	155 ± 11.2
Height z-score	0.19 ± 0.26*	0.8 ± 1.2	0.8 ± 1*
BMI	20.5 ± 2.1$^{*\#}$	25.4 ± 1.1*§	30.3 ± 4.4$^{\#\S}$
BMI z-score	0.67 ± 0.3*	1.92 ± 0.7*	2.9 ± 0.6*
Fat mass, FM (%)	17.8 ± 5.1*	34.4 ± 9.3*	40.1 ± 5.9*
Lean mass, Kg	44.1 ± 9.7	45.0 ± 11.3	43.8 ± 10.8
Trunk fat, TF (%)	15.9 ± 3.9*	32.2 ± 8.7*	39.4 ± 11.2*
Systolic blood pressure, mm Hg	116.2 ± 10.8	113.2 ± 9.1	111.3 ± 1.6
Diastolic blood pressure, mmHg	61.6 ± 5.2$^{*\#}$	68.2 ± 6.4*	65.8 ± 7.5$^{\#}$

Pubertal stage: $^{*}p = 0.003$ N vs O. Height z-score: $^{*}p = 0.03$ N vs O. BMI: $^{*}p = 0.02$ N vs OW; $^{\#}p < 0.0001$ N vs O; $^{\S}p = 0.018$ OW vs O. BMI z-score: $^{*}p < 0.0001$ N vs O and OW. FM %: $^{*}p < 0.0001$ N vs O and OW. TF: $^{*}p < 0.0001$ N vs O and OW. DBP: $^{*}p = 0.04$ N vs OW $^{\#}p = 0.02$ N vs O. CNP: $^{*}p = 0.0003$ N vs OW $^{\#}p < 0.0001$ N vs O.

2.4. Endothelium-dependent vasodilation

Peripheral endothelium-dependent vasodilator capacity was estimated assessing reactive hyperemia index (RHI) by means of an EndoPAT system (Itamar Medical Ltd., Cesarea, Israel) as previously described [30,31]. Briefly, the subject sat in a reclining chair with the hands at heart level and was propped in a comfortable position so that the fingers were hanging freely. Fingertip probes were placed on both index fingers and pulse wave amplitudes were recorded for the duration of the entire study that consists of: 5-minute baseline recording; 5-minute occlusion of non-dominant arm using a BP cuff inflated to 40 mmHg above systolic pressure; rapid deflation of BP cuff (followed by reactive, flow-mediated hyperaemia) and pulse wave amplitudes recording for at least further 5 min. An integrated software program compares the ratio of arterial pressure waves in the two fingers before the occlusion and after the deflation to calculate the reactive RHI score as the ratio of the average pulse wave amplitude, measured over 60 s starting 1 min after cuff deflation, to the average pulse wave amplitude measured at baseline. This ratio is normalized to the concurrent signal from the contralateral finger to correct for changes in systemic vascular tone.

2.5. Natriuretic peptides determinations

2.5.1. CNP assay

As previously reported [8,32], CNP was directly measured in plasma by a specific RIA (Phoenix Pharmaceuticals, Belmount, CA, USA). Each sample was assayed in duplicate in an ice bath. A control sample, prepared by using known amounts of CNP standard added to the assay buffer and stored in aliquots to −80 °C, was assayed in each run for quality control purpose.

2.5.2. NT-proBNP assay

Plasma levels of NT-proBNP were measured by a fully automated electrochemiluminescence immunoassay on an Elecsys® 2010 analyzer (Roche Diagnostics GmbH, Mannheim) [33].

2.5.3. MR-proANP assay

MR-proANP was measured in 50 μL of plasma by a Time-Resolved Amplified Cryptate Emission (TRACE) technology assay, using a kit designed for automated sandwich immunofluorescent assay of MR-proANP (KRIPTOR:BRAHMS AG). The KRYPTOR MR-proANP has a detection range of 2.1–10,000 pmol/L.

Within-assay variability was evaluated using two samples at different concentrations and both resulted <10%: $CK_1 = 31.94 \pm 0.58$ pmol/L (n = 5 duplicate assays, CV = 4%) and $CK_2 = 26.5 \pm 0.28$ pmol/L (n = 5 duplicate assays, CV = 2.4%). Accuracy was evaluated by dilution tests and a linearity of the response was observed. Two control samples were assayed in each run for quality control.

2.6. Statistics

All sample values and other data for quality control of 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]. Data are expressed as mean ± SD. Variables with a skewed distribution are given as median [25th - 75th percentile range] and were Log transformed for use in statistical analyses. A p-value < 0.05 was considered significant.

To detect differences among groups, data were analyzed by ANOVA test, followed by Fisher's test. Relations between variables were assessed by univariate and multivariable linear regression analysis.

After building the receiver-operating characteristic (ROC) curves for CNP, NT-proBNP and MR-proANP, the ROC area under the curve (AUC) was evaluated to analyze the association of individual biomarkers with the presence of obesity. A multivariable logistic regression analysis was also used to identify dependent and independent parameters.

Statistical analysis was carried out by using a dedicated statistical software (JMP 12 for MAC; SAS Institute, Inc., Cary, NC, USA). Graphs were drawn using Prism 6.0 for MAC (GraphPad Software, La Jolla, CA, USA), in addition to JMP 12.

3. Results

As expected, O and OW adolescents had higher values of BMI, BMI z-score and fat mass, both FM% and TF%, than normal weight subjects. Height z-score was significantly increased only in O and diastolic blood pressure was higher in O and OW subjects in comparison with normal weight adolescents (Table 1a). Finally Tanner Score of O resulted significantly higher than that of N (Table 1a).

As reported in Table 1b, fasting plasma glucose, HbA1c, HDL cholesterol and triglycerides were similar among groups, while CRP, insulin, HOMA-IR, total cholesterol and LDL cholesterol resulted significantly higher in OW/O than in normal weight adolescents. Our OW/O subjects showed higher amounts of skin AGEs than N (Table 1b). In univariate analysis, skin AGEs resulted significantly associated with FM% ($R = 0.87$; $p < 0.0001$), TF% ($R = 0.88$; $p < 0.0001$), circulating levels of cholesterol ($R = 0.46$; $p = 0.0004$), LDL ($R = 0.48$; $p = 0.0002$) and triglycerides ($R = 0.31$; $p < 0.021$). We also observed significantly lower RHI values in OW and O subjects in comparison with N. Interestingly, we found that RHI values correlated inversely with serum CRP levels ($R = -0.68$; $p < 0001$) and skin AGEs ($R = -0.55$; $p < 0001$).

CNP plasma levels resulted progressively and significantly reduced in OW and in O with respect to N (Fig. 1), while NT-proBNP and MR-proANP did not change significantly among groups (Table 1a).

Table 2 summarizes correlations (univariate model) between circulating LogCNP and some anthropometric and body composition data, skin AGEs, RHI, and endocrine-metabolic variables. We found that LogCNP values correlated significantly and inversely with BMI z-score and TF%, while directly with height (cm). We also observed a significant inverse association between LogCNP and FM%, but not with lean mass. We also found a significant inverse correlation between blood LogCNP and circulating levels of CRP, insulin, total cholesterol, LDL, and triglycerides, in addition to an inverse relationship with skin AGEs and a direct correlation with RHI. Finally, LogCNP was inversely associated with LogNT-proBNP and LogMR-proANP values.

Including LogCNP, as dependent variable, in a multiple regression analysis incorporating some metabolic, inflammatory and clinical parameters (total cholesterol, LDL, triglycerides, insulin, AGEs, CRP, Tanner score, height, SBP and DBP) as independent variables, we found that skin AGEs, serum CRP levels, Tanner score and DBP were independent determinants of circulating LogCNP, while total and LDL cholesterol, triglyceride, insulin, height and SBP values were not (Tables 3a, 3b). After substituting insulin with HOMA-IR in the same equation, we also observed that it was not an independent determinant of circulating LogCNP [Intercept: B(SE) 1.78(0.58); T: 3.07; $p = 0.0037$. HOMA-IR: B(SE) −0.018(0.01); T: −1.58; $p = 0.12$].

As far as the multiple regression analysis models are considered, LogCNP (dependent variable) was found significantly ($R = 0.803$, $p < 0.0001$) and positively associated with age, but negatively with sex (F = 2, M = 1) and obesity (subjects analyzed according to obesity, N = 0, OW = 1, O = 2) (Table 4, upper panel). On the contrary, LogNT-proBNP was found significantly and negatively ($R = -0.502$, $p < 0.0001$) associated with age and positively with sex, but there was no association with obesity (Table 4, middle panel). Finally LogMR-proANP was significantly and negatively ($R = -0.394$, $p < 0.0057$) associated with age, but not with sex and obesity (Table 4, lower panel).

Using ROC analysis, considering OW and O as a whole, the risk of obesity resulted significantly ($p \ll 0.0001$) associated with CNP values (AUC = 0.9724), with a sensitivity value of 0.898, specificity value 0.963, and a total accuracy of 85.4% at the cut-off of 8.6 ng/L (Fig. 2a)

Table 1b
Laboratory data of adolescents at normal weight, overweight and with obesity (values as mean ± SD). [ʃ]: skewed variables expressed as median [25th and 75th percentiles].

	Normal weight subjects N = 27	Overweight subjects OW = 10	Subjects with obesity O = 45
Blood glucose (mg/dL)	80.2 ± 7.6	84.3 ± 3.8	86.5 ± 10.9
ʃCRP (mg/dL)	0.016 [0.001–0.03]*	0.19 [0.016–0.27]*	0.35 [0.2–0.47]
HbA1c (mmol/mol)	30.1 ± 3.8	32.4 ± 4	32.7 ± 4.8
Insulin (uU/mL)	8.0 ± 4.8*#	21.1 ± 8.6*	20.0 ± 10.6#
HOMA-IR	1.5 ± 0.6*	3.3 ± 1.1	4.1 ± 2.5*
Cholesterol (mg/dL)	134.4 ± 38.9*	139.0 ± 46	168.2 ± 38.6*
HDL (mg/dL)	47.3 ± 11.7	37.0 ± 13.5	44.6 ± 8.9
LDL (mg/dL)	72.9 ± 29.7*	87.3 ± 32§	104.1 ± 30*§
ʃTriglycerides (mg/dL)	47 [25–372]	56 [31–98]	90 [41–287]
AGEs (AF, arbitrary units)	1.1 ± 0.32*	1.7 ± 0.4	1.97 ± 0.4
RHI	2.1 ± 0.2*	1.6 ± 0.4	1.4 ± 0.3
CNP, ng/L	13.21 [7.6–37.8]*#	4.79 [3.29–21.15]*	3.81[1.55–13.4]#
NT-proBNP, ng/L	25.67 [5–115.1]	42.39 [8.49–208.9]	41.60 [5–211.2]
MR-proANP, pmol/L	27.87 [14.42–49.95]	32.52 [13.55–46.27]	29.60 [13.1–74.9]

CRP: *p = 0.0001 N vs OW and O; *p = 0.0001 OW vs O. Insulin: *p = 0.03 N vs OW #p < 0.0001 N vs O. HOMA-IR: *p < 0.0001 N vs O. Cholesterol: *p = 0.003 N vs O. LDL: *p = 0.0005 N vs O; §p = 0.024 OW vs O. Ages: *p < 0.0001 N vs O and p = 0.0068 N vs OW. RHI: *p = 0.0085 N vs OW and p < 0.0001 N vs O.

In Fig. 2b the logistic ROC plot was reported. The risk of obesity was significantly (p = 0.0235) associated also with NT-proBNP values (AUC = 0.683), with a sensitivity of 0.845 and a specificity of 0.482 at the cut-off of 24.9 ng/L. On the contrary, obesity showed only an association trend (p = 0.0562) with MR-proANP values (AUC = 0.633), with a sensitivity value of 0.776 and a specificity value of 0.518, respectively, at the cut-off of 26.5 pmol/L. The discrimination analysis, using the ROC model, showed that the risk to have a deficient peripheral endothelium-dependent vasodilator capacity (estimated by RHI value) was significantly ($p \ll 0.0001$) associated with CNP values (AUC = 0.886). However, in a multivariate logistic regression analysis, only LogCNP (p = 0.006) and age (p = 0.0355) associated significantly with obesity, while sex showed only a trend for association (p = 0.0513). On the contrary NT-proBNP and MR-proANP were not associated with the presence of obesity (Table 5).

4. Discussion

In the present study, we evaluated plasma CNP in young adolescents at normal weight and with obesity. Our data indicated that CNP plasma levels were progressively reduced in OW/O in comparison with N (Fig. 1). This observation confirmed our preliminary data of reduced plasma levels of this peptide in children with obesity [8]. Compared with our previous study, in the present one we increased the number of O/OW adolescents and added a matched control group. This allowed us to use multivariable linear and logistic regression analyses. We have also increased the scientific information of our study by assessing several cardiovascular and metabolic biomarkers, including: plasma CRP, plasma NT-proBNP, plasma MRproANP, skin AGEs, and peripheral endothelium-dependent vasodilator capacity by RHI.

Our data indicated that OW/O subjects had significant higher skin AGEs and blood CRP values than normal weight counterparts. Interestingly, including metabolic parameters in a multiple regression model, we observed that both skin AGEs and blood CRP were independent determinants of low circulating CNP levels. Since our OW/O subjects had normal levels of blood glucose and HbA1c, the increase in skin AGEs may be due mainly to an external source such as eating food reach in AGEs [35]. The relationship between skin AGEs and circulating levels of total cholesterol and LDL may also reflect an incorrect food eating of OW/O subjects. The direct relationship between TF% and skin AGEs confirmed the increased risk of vascular disease due to accumulation of abdominal fat and AGEs in OW/O subjects. AGEs are, in fact, involved in oxidative stress at vascular level. Thus, their accumulation into tissues, including endothelia, may lead to local inflammation and endothelial damage, contributing to reduce circulating CNP levels. Similarly, it was previously reported that inflammatory stress response rapidly reduced CNP production in inflamed skeletal tissue in young children [36]. Childhood obesity is associated with an increased risk of endothelial dysfunction and atherosclerosis, possibly mediated by an increase in angiogenesis and inflammation [36]. Considering OW/O and N as a whole, we found that RHI correlated significantly and inversely with skin AGEs and blood CRP levels, suggesting a noxious effect of AGEs accumulation on endothelial tissue leading to endothelial dysfunction.

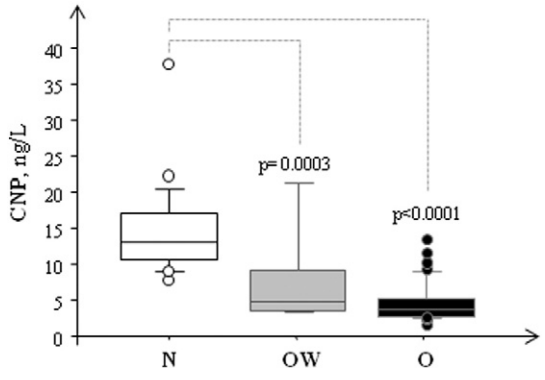

Fig. 1. CNP plasma levels in normal weight (N, white box), overweight (OW, gray box) and with obesity (O, black box) subjects. Each box consists of 5 horizontal lines displaying 10th, 25th, 50th (median), 75th and 90th percentiles of the variable. All values above 90th percentile and below 10th percentile are plotted separately.

Table 2
Univariate analysis between circulating CNP levels and anthropometric data, body composition, skin AGEs, and endocrine-metabolic factors.

Response variable	Regressors	R	p
LogCNP	Age (yrs.)	0.52	<0.0001
	Height (cm)	0.42	0.0002
	Height z-score (N)	0.53	0.004
	Height z-score (OW-O)	0.017	n.s.
	Tanner Score	0.464	0.0002
	BMI z-score	-0.94	<0.0001
	Fat mass (%)	-0.92	<0.0001
	Trunk fat (%)	-0.94	<0.0001
	LogCRP (mg/dL)	-0.76	<0.0001
	Skin AGEs (AF)	-0.88	<0.0001
	RHI	0.64	<0.0001
	Insulin (μU/mL)	-0.62	<0.0001
	Total cholesterol (mg/dL)	-0.52	<0.0001
	LDL cholesterol (mg/dL)	-0.54	<0.0001
	LogTriglycerides (mg/dL)	-0.42	0.015
	LogNT-proBNP (ng/L)	-0.417	0.0002
	LogMR-proANP (pmol/L)	-0.284	0.01

Values of CNP, CRP, Triglycerides and NT-proBNP were logarithmically transformed before analysis.

Table 3a
Metabolic data as independent determinants of circulating CNP (response variable).

	B(SE)	t	p
Intercept	1.27(0.16)	7.57	*<0.0001*
LogCRP	−0.13(0.03)	−4.44	*<0.0001*
Ages	-0.37(0.05)	−7.70	*<0.0001*
Cholesterol	−0.002(0.001)	−1.58	0.12
LogTriglycerides	0.16(0.09)	1.66	0.10
LDL	0.001(0.001)	0.80	0.42
Insulin	−0.002(0.002)	−0.93	0.35

Values of CNP, CRP and Triglycerides were logarithmically transformed before analysis.

Another mechanism, contributing in lowering blood CNP levels, might be its increased clearance due to the overexpressed natriuretic peptide receptor-C (NPR—C) observed in subject with obesity [37]. Binding of CNP to NPR-C promotes internalization of the CNP/NPR-C complex and enzymatic degradation of the peptide [37]. The lack of association between BMI and markers of cardiac endocrine function, such as NT-proBNP and MR-proANP, may suggest that OW/O subjects were still in a preclinical stage of atherosclerosis. In fact, circulating levels of NT-proBNP and MR-proANP were similar in OW/O subjects and in control group, suggesting that they had a normal cardiac endocrine function [14].

For the first time an independent association between CNP and obesity in a population of Caucasian adolescents was observed. The analysis was performed on a small number of overweight children and further studies, on a large number of them, are necessary to confirm this result. However, analyzing data with ROC analysis, the presence of obesity was associated with lower CNP plasma levels, with a cut-off <9 ng/L.

NT-proBNP and MR-proANP showed only an association trend with RHI score, while a significant association was found between CNP and RHI. These results suggest that CNP may play a more important role than BNP and ANP related peptides, as risk marker of obesity, in addition to its involvement in adipogenesis and endothelial dysfunction.

A limitation of present study was the relative low number of subjects at normal weight and with obesity studied, preventing us to include no more than 7 predictive variables in the multivariate models of regression analyses.

5. Conclusion

Our results confirmed that CNP levels were significantly lower in OW/O subjects than normal weight adolescents. We also showed that skin AGEs and circulating CRP levels were independent determinants of low blood CNP values found in OW/O. Interestingly, a reduction in CNP values associated with impaired peripheral endothelium-dependent vasodilator capacity, suggesting a role of CNP in the pathophysiological mechanisms related to endothelial dysfunction.

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Table 3b
Clinical data as independent determinants of circulating CNP (response variable).

	B(SE)	t	p
Intercept	1.002(0.45)	2.22	*0.031*
Tanner score	0.12(0.03)	3.72	*0.0005*
Height z-score	−0.04(0.03)	−1.22	0.22
SBP	−0.003(0.003)	0.99	0.32
DBP	−0.01(0.004)	−2.96	*0.0047*

Table 4
Multiple regression analysis for CNP, NT-proBNP and MR-proANP.

LogCNP	B(SE)	t	p
Intercept	1.029(0.19)	5.35	*<0.0001*
Age, years	0.017(0.01)	1.54	0.127
Sex, M/F	−0.098(0.04)	−2.11	*0.037*
Obesity class	−0.243(0.03)	−8.52	*<0.0001*
LogNT-proBNP			
Intercept	19.04(0.34)	5.51	*<0.0001*
Age, years	−0.056(0.02)	−2.84	*0.0058*
Sex, m/f	0.205(0.08)	2.38	*0.0198*
Obesity class	0.059(0.05)	1.148	0.254
LogMR-proANP			
Intercept	1.688(0.0.13)	12.48	*<0.0001*
Age, years	−0.021(0.008)	−2.72	*0.0081*
Sex, M/F	0.037(0.03)	1.11	0.270
Obesity class	0.007(0.02)	0.34	0.728

Independent variables sex (F = 2, M = 1), age, and the obesity class according to obesity (N = 0, OW = 1, O = 2).

Disclosure

None to disclose.

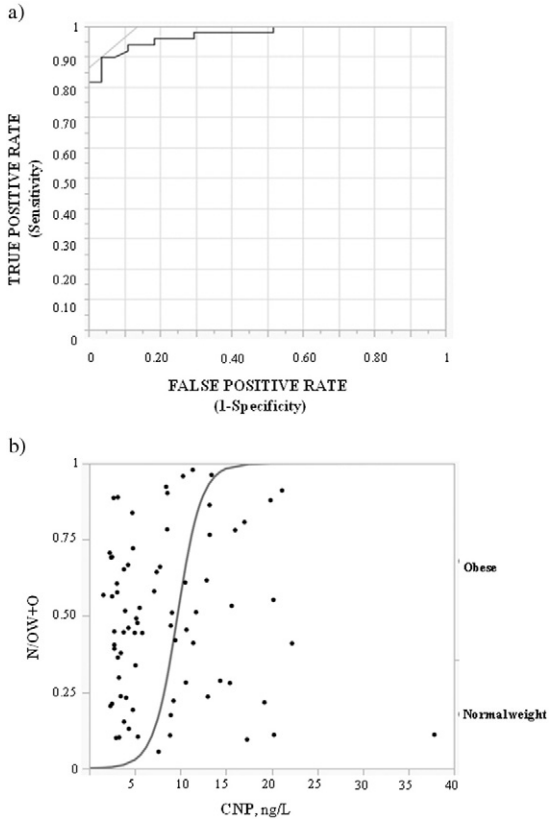

Fig. 2. Receiver-operating characteristic (ROC) curve analysis performed to evaluate the risk of obesity associated to CNP values. The point of the curve indicating the best cut-off value, corresponding to 8.6 ng/L, is reported in the figure a); in b) logistic curve was reported.

Table 5
Multivariable logistic regression analysis for presence of obesity.

Log CNP	Coefficient	STD error, SE	Coefficient/STD error	Chi-square	p-Value	Exp (coefficient)
Obesity (constant)	45.570	19.375	2.352	5.532	0.0187	6.179E19
Log CNP	−19.408	7.067	−2.746	7.543	0.006	3.725E−9
Log NT-proBNP	2.974	3.303	0.900	0.810	0.3680	19.561
Log MR-proANP	−9.941	8.334	−1.193	1.423	0.2329	4.818E−5
Age, years	−0.919	0.437	−2.102	4.419	0.0355	0.399
Sex, M/F	−3.163	1.623	−1.949	3.799	0.0513	0.042

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