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ORIGINAL CONTRIBUTION

Mid-regional-pro-adrenomedullin plasma levels are increased in obese adolescents

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Abstract
Purpose Recently, adrenomedullin (ADM) was defined as a new member of the adipokine family. ADM secreted by adipocytes, through its vasodilator and antioxidant actions, might be protective against metabolic syndrome-associated cardiovascular complications. The aim of the study was to assess plasma mid-regional (MR)-proADM levels in obese adolescents compared to normal-weight subjects and its relation with BMI, body composition and metabolic indices.
Methods Plasma MR-proADM was measured in 32 healthy adolescents [BMI *z*-score (mean ± SEM) = 0.6 ± 0.09 and 0.8 ± 0.07 in females and males, respectively] and in 51 age-matched obese adolescents [BMI *z*-score (mean ± SEM) = 2.8 ± 0.12 and 2.9 ± 0.08 in female and males, respectively] by a time-resolved amplified cryptate emission technology assay.
Results Plasma MR-proADM levels resulted significantly higher in obese than in normal-weight adolescents (MR-proADM: 0.33 ± 0.1 vs 0.40 ± 0.1 nmol/L, $p < 0.0001$). Using univariate analysis, we observed that MR-proADM correlated significantly with BMI *z*-score ($p < 0.0001$), fat mass ($p < 0.0001$), circulating insulin ($p < 0.004$), HOMA-IR ($p < 0.005$), total cholesterol ($p < 0.03$) and LDL-cholesterol ($p < 0.05$). Including MR-proADM as response variable and its significant correlates into a multiple regression analysis, we observed that fat mass ($p = 0.014$) and BMI *z*-score ($p = 0.036$) were independent determinants of circulating MR-proADM.
Conclusions Our study shows for the first time that obese adolescents have higher circulating levels of MR-proADM compared with normal-weight, appropriate controls suggesting its important involvement in obese patients.

Keywords MR-proADM · Obesity · Adolescents · Adipokine

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Introduction

Adipose tissue is known to express and secrete a variety of bioactive substances called adipokines that act at both local and systemic levels [1]. Studies on adipokines and other biomarkers of obesity have become important in obesity research, and adrenomedullin (ADM) was also defined as a new member of adipokine family [1, 2].

Adrenomedullin is a vasoactive peptide expressed and secreted by a variety of tissues, including adrenal medulla, heart, lung, kidney and pancreatic islets [3, 4], vascular smooth cells, immune cells and several endocrine glands [2, 3]. Plasma ADM concentrations were found elevated in patients with hypertension [5], renal failure [5], heart failure [6–8], shock [9] and diabetes mellitus [10]. ADM exhibits protective effects on heart and vasculature by inducing vasodilation and natriuresis, improving vascular endothelial function, increasing

cardiac output and inhibiting left-ventricular remodelling and apoptosis [11, 12].

In addition, it was reported that adult obese individuals had increased amounts of ADM in both plasma and adipose tissue, compared to lean subjects [2, 13].

Metabolic syndrome, commonly found in obese subjects, is a constellation of metabolic risk factors [14], and many substances secreted by adipocytes have been implicated in its development [15]. One possible role of ADM secreted by adipocytes may be linked to its vasodilator and antioxidant action [2]. The ADM precursor gene (pre-proADM) is transcriptionally induced by insulin, hypoxia and inflammatory stimuli [16, 17]. Acute hyperinsulinemia has been also associated with increased circulating plasma ADM levels in diabetic patients [18] and in subjects with uncomplicated obesity [19]. Since ADM expression is increased in adipose tissue of obese subjects, one possibility is that, in these subjects, the adipose tissue itself may be the source of elevated plasma ADM [2].

Although the prevalence of obesity is increasing steadily and dramatically, representing a worldwide health problem [20], there are no data regarding this peptide in childhood obesity.

ADM has a short half-life, and the accurate assessment of its circulating levels is challenging [4]. In the last years, an assay to determine circulating mid-regional-pro-adrenomedullin (MR-proADM) concentration has become available [21]. This peptide has the advantage of a longer half-life, lack of bioactivity and protein binding, which makes it more suitable for daily practice [22]. Therefore, circulating MR-proADM reliably reflects the amount of released ADM [21]. The aim of the present study was to investigate whether plasma MR-proADM behaved differently in obese children and adolescents in comparison with normal-weight age- and sex-matched controls.

Materials and methods

Subjects and plasma samples

Eighty-three Caucasian subjects were enrolled in the study: 51 obese adolescents (age 12.50 ± 0.41 years) without cardiac dysfunction referred as outpatients to the Unit of Pediatric Endocrinology and Diabetes, Department of Pediatrics, University of Pisa, Italy, and 32 age- and sex-matched normal-weight subjects (age 12.8 ± 0.2 years) as controls. BMI was calculated using the formula weight (kg)/height $(m)^2$ [23]. Obesity was defined according to national BMI reference data specific for age and sex [24]. We used the same national reference data [24] to calculate *z*-score of height, weight and BMI. Normal-weight adolescents had a BMI *z*-score (mean ± SEM) of 0.6 ± 0.09 in females and 0.8 ± 0.07 in males, and BMI *z*-score was 2.8 ± 0.12 and 2.9 ± 0.08 in female and male obese subjects. As in the examined population BMI *z*-score showed a skewed distribution, it was logarithmically transformed and summarized as median [interquartile range] for statistical purpose. Table 1 summarizes the clinical details of participants in the study, subdivided into male and female.

Table 1 Clinical characteristics of obese and normal-weight, healthy adolescents

	Obese subjects Mean ± SEM Median [interquartile range]				Controls Mean ± SEM Median [interquartile range]				Obese versus controls	
									Male	Female
	Male	Range	Female	Range	Male	Range	Female	Range	*p*	*p*
Sex (*n*)	24	–	27	–	19	–	13	–		
Age (years)	11.9 ± 0.3	8.9–17	12.1 ± 0.3	9–16	12.9 ± 0.1	12–14	12.7 ± 0.4	8–15	n.s.	n.s.
Pubertal stage	1–4	1–4	1–4	1–4	1–4	1–4	1 - 4	1–4		
Height (cm)	156.1 ± 1.9	135–191	156.2 ± 1.7	137–172	157.9 ± 1.0	150–164	154.5 ± 2.1	131–163	n.s.	n.s.
Height (*z*-score)	0.98 ± 0.1	−0.9 to 3.2	0.97 ± 0.2	−1.0 to 3.0	0.4 ± 0.08	−0.2 to 1.3	0.2 ± 0.05	−0.2–0.7	<0.02	<0.004
Weight (kg)	71.4 ± 2.4	47.8–191	74.4 ± 3.9	42.7–125	50.0 ± 0.8	44–56.5	48.8 ± 1.8	31.3–56	<0.0001	<0.0001
Weight (*z*-score)	2.2 ± 0.09	1.0–3.1	2.2 ± 0.12	1.16–3.6	0.55 ± 0.03	0.3–0.87	0.49 ± 0.06	0.19–1.0	<0.0001	<0.0001
BMI *z*-score	1.0 [0.3]	0.5–1.4	0.9 [0.4]	0.7–1.4	0.2 [0.8]	−1.6 to 0.2	−0.3 [0.7]	−2.3 to 0.3	<0.0001	<0.0001
Fat mass, FM (%)	33.1 ± 1.0	24.2–44.1	40.4 ± 1.7	33.3–58.2	15.0 ± 1.2	7.2–26.3	21.8 ± 1.0	13.9–28.7	<0.0001	<0.0001
SBP (mmHg)	111 ± 2.0	94–146	113 ± 2.3	95–128	116 ± 0.9	102–116	106 ± 1.8	96–115	n.s.	<0.001
SBP percentile	65 ± 3.6	15–100	71 ± 4.6	15–97	58 ± 3.9	23–76	44 ± 4.3	23–78	n.s.	<0.001
DBP (mmHg)	63 ± 1.3	50–80	67 ± 1.4	57–83	62 ± 1.9	55–67	61 ± 1.7	53–64	n.s.	<0.001
DBP percentile	49 ± 3.2	13–93	58 ± 3.9	27–96	42 ± 2.3	26–63	37 ± 3.1	12–58	n.s.	<0.0005

SBP systolic blood pressure, *DBP* diastolic blood pressure

Body composition was measured using the Tanita BC-418 Segmental Body Composition Analyzer (Tanita Corporation, Tokyo, Japan) [25].

Blood pressure was measured by the same investigator at the left brachial artery (Omron, Kyoto, Japan) using a cuff appropriate for arm dimensions. Results were compared with height-, age- and sex-specific reference values to calculate the proper percentile and assess whether a condition of pre- or hypertension was present [26].

Blood samples were collected in all the subjects by venipuncture in the morning after overnight fasting. Blood samples for MR-proADM 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 was stored frozen at −80 °C in 1-mL aliquots in polypropylene tubes until assay, performed within 1 month. Blood samples for blood glucose and lipid assays were collected in lithium/heparin-containing vials, and 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.

Biochemical assays

A Cobas Integra 400 analyzer (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 triglycerides (Cobas Integra 400 Triglycerides; 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.

MR-proADM assay

MR-proADM was measured in 50 μl of plasma by a time-resolved amplified cryptate emission (TRACE) technology assay [27], using a kit designed for automated sandwich immunofluorescent assay of MR-proADM (KRYPTOR: BRAHMS AG). The KRYPTOR MR-proADM has a detection range of 0.05–100 nmol/L.

Within-assay variability was evaluated using two samples at different concentrations and both resulted <10 %: CK1 = 0.297 ± 0.1 nmol/L (n = 5 duplicate assays, CV = 8.6 %) and CK2 = 0.348 ± 0.009 nmol/L (n = 5 duplicate assays, CV = 5.7 %) nmol/L. 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.

BNP and NTproBNP assay

To verify that our obese population had not relevant cardiovascular involvement, we measured BNP and NTproBNP plasma levels. Circulating BNP was assessed using the fully automated Access platform (Triage BNP reagents, Access Immunoassay Systems, REF 98200; Beckman Coulter, Inc, Fullerton, CA, USA) as previously described [28]. NTproBNP concentrations were measured by a fully automated electrochemiluminescence immunoassay on an Elecsys® 2010 analyzer (Roche Diagnostics GmbH, Mannheim) [29].

Statistical methods

Results are expressed as mean ± SEM, unless noted otherwise. Variables with skewed distribution (MR-proADM, BNP, NTproBNP, insulin, HOMA-IR and BMI z-score) were logarithmically transformed for parametric statistical analysis. Student t test was used to compare continuous variables. Univariate regression analysis was used to evaluate the relationships between variables. Multiple linear regression analysis was used to identify the independent associations of MR-proADM with its significant univariate correlates. Statistical tests were two-sided, and significance was set at a value of $p < 0.05$. Statistical analysis was performed by StatView 5.01 and JMP software, version 11 (SAS Institute Inc, Cary, NC, USA).

Results

Table 1 summarizes some clinical characteristics of the examined population.

Since blood pressure changes according to height, age and sex, we analyzed the baseline parameters separately in male and female subjects. Obese adolescents, both males and females, were higher than non-obese counterparts, as shown by the increased height z-score. As expected, they also had a significant increase in fat mass percentage in comparison with normal-weight controls, as assessed by body composition. As regards blood pressure, we observed that obese girls had significantly higher blood pressure than

Table 2 Biochemical characteristics of obese and healthy adolescents

	Obese subjects Mean ± SEM Median [Interquartile range]	Range	Normal-weight controls Mean ± SEM Median [Interquartile range]	Range	*p*
Glycemia (mg/dl)	86 ± 1.3	71–103	78 ± 1.5	46–99	<0.0006
Insulin (uU/mL)	2.8 [0.77]	1.33–4.0	1.94 [0.58]	0.99–2.6	<0.0001
HOMA-IR	1.18 [0.91]	−0.56 to 2.43	0.41 [0.67]	−0.6 to 1.0	<0.0001
Cholesterol (mg/dl)	173 ± 4.1	112–250	131 ± 8.3	68–195	<0.0001
HDL (mg/dl)	48 ± 1.5	30–74	45 ± 3.0	19–70	n.s.
LDL (mg/dl)	105 ± 4	53–181	73 ± 6.3	37–160	<0.0001
Triglycerides (mg/dl)	4.29 [0.67]	3.29–5.65	3.92 [0.62]	3.2–5.9	<0.04
BNP (ng/L)	20.6 ± 2.3	–	15.4 ± 1.8	1–46	n.s.
NTproBNP (ng/L)	50.2 ± 5.13	–	39.6 ± 6.3	5–391	n.s.

their non-obese counterparts. Such a difference was not present in males (Table 1).

Table 2 summarizes the laboratory characteristics of obese and healthy adolescents enrolled in the study. Obese subjects showed a significant increase in circulating levels of glucose, insulin, total cholesterol and LDL-cholesterol. The mean value of HOMA-IR was also higher in obese than in normal-weight adolescents.

Plasma MR-proADM levels resulted significantly higher in obese than in normal-weight adolescents (MR-proADM: 0.40 ± 0.1 vs. 0.33 ± 0.1 nmol/L, $p < 0.0001$, Fig. 1). In addition, while circulating levels of MR-proADM correlated directly and significantly with age in normal-weight individuals ($R = 0.39$; $p = 0.04$), this relationship disappeared in obese subjects ($R = 0.1$; $p = 0.95$).

Using univariate analysis, we observed that MR-proADM correlated significantly with BMI *z*-score, fat mass, circulating insulin, HOMA-IR, total cholesterol, LDL-cholesterol, but not with triglycerides and blood pressure (Table 3). Including MR-proADM as response variable and its significant correlates into a multiple regression analysis, we observed that fat mass and BMI *z*-score were independent determinants of circulating MR-proADM (Table 4).

BNP and NTproBNP plasma levels resulted in normal value range [30, 31] and are similar in both controls and obese children (Table 2).

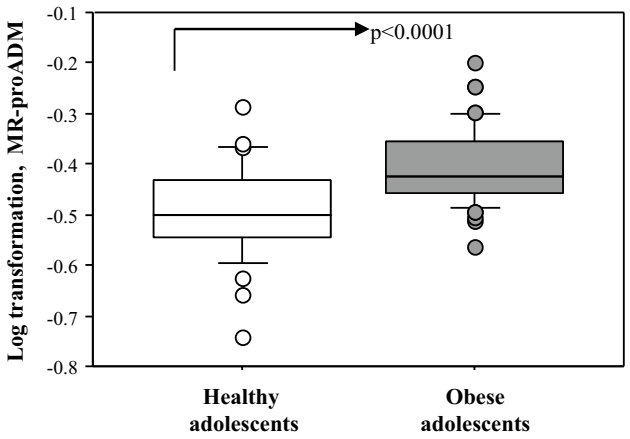

Fig. 1 MR-proADM plasma levels in normal-weight and obese adolescents. *White box*: healthy adolescents ($n = 32$); *gray box*: obese adolescents ($n = 51$), $p < 0.0001$. *Each box* consists of 5 *horizontal lines* displaying 10th, 25th, 50th (median), 75th and 90th percentiles of the variable. All values above 90th percentile and below 10th percentile are *plotted* separately

Table 3 Univariate analysis between circulating MR-proADM, body composition and metabolic indices

Response variable	Regressors	R	*p*
MR-proADM	BMI *z*-score	0.54	*<0.0001*
	Fat mass (%)	0.56	*<0.0001*
	Lean mass (Kg)	−0.86	0.41
	Insulin	0.34	*<0.004*
	Homa-IR	0.33	*<0.005*
	Systolic blood pressure	0.008	0.94
	Diastolic blood pressure	0.22	0.06
	Total cholesterol	0.28	*<0.03*
	LDL-cholesterol	0.26	*<0.05*
	Triglycerides	0.098	0.45

Values of MR-proADM, BMI *z*-score, insulin, HOMA-IR and triglycerides were logarithmically transformed before analysis

Discussion

For the first time, the results of this study showed significantly increased circulating levels of MR-proADM in obese adolescents, confirming that ADM plasma concentrations are increased as previously found in obese adults [2, 13]. As expected, univariate analysis showed a clear, direct relationship between circulating MR-proADM and

Table 4 Independent determinants of circulating MR-proADM (response variable)

	B (SE)	*t*	*p*
k	−0.77 (0.37)	−2.07	0.04
Fat mass (%)	0.009 (0.003)	2.57	0.014
BMI *z*-score	0.08 (0.03)	2.19	0.035
Insulin	−0.32 (0.22)	−1.41	0.16
HOMA-IR	0.19 (0.21)	0.94	0.35
Total cholesterol	0.0004 (0.001)	0.30	0.76
LDL-cholesterol	−0.0006 (0.001)	−0.40	0.7
Cumulative corrected $R^2 = 0.3$			0.0028

Values of MR-proADM, BMI *z*-score, insulin and HOMA-IR were logarithmically transformed before analysis

indices of fat tissue, but not with lean mass (Table 3), suggesting that adipose tissue is a major source of ADM in pediatric as well as in adult obese patients [16, 32]. This observation is reinforced by the results we obtained from multiple regression analysis. It showed that both fat mass and BMI *z*-score were independent determinants of MR-proADM in a model that also included insulin, HOMA-IR, total cholesterol and LDL-cholesterol (Table 4). In addition, our finding, showing that the direct relationship between MR-proADM and age, observed in normal-weight subjects, is lost in obese individuals, fits well with the hypothesis that adipose tissue is a major source of the peptide. Changes in circulating levels of MR-proADM during adolescence possibly reflect changes in body composition, endocrine and metabolic milieu. It might be that, in obese adolescents, the excess in fat mass causes MR-proADM overproduction, drowning out its age-related changes. The higher MR-proADM plasma levels found in obese adolescents are unlikely to be attributable to cardiovascular alterations, since both BNP and NTproBNP, considered sensitive biomarkers of cardiac dysfunction used in the diagnostic and prognostic staging of chronic and acute cardiac conditions across all ages [33], resulted in normal range and comparable between obese and normal-weight adolescents.

The pathophysiological role of ADM secreted by adipose tissue in obese subjects is still speculative. One possibility might be linked to both the antioxidant and strong vasodilator effects of the peptide to antagonize metabolic derangement and hypertension associated with obesity. It is known that obesity is characterized by mild systemic inflammation, increase in circulating cytokines [34] and hypoxia within the adipose tissue [35]. Thus, ADM release into adipose tissue should antagonize, at least in part, the deleterious effects of tissue hypoxia and inflammation. Our observation of a direct relationship between circulating levels of MR-proADM and blood insulin, insulin resistance assessed by HOMA-IR, and circulating levels of total cholesterol and LDL-cholesterol supports the hypothesis that peptide release might be triggered by the metabolic derangement linked to adipose tissue in obese subjects. However, some experimental studies seem to suggest quite different effects of ADM contributing to hypertension and diabetes mellitus in obese subjects, through its effect on brain [36, 37] and pancreas [38, 39].

In conclusion, our study shows for the first time that obese adolescents have higher circulating levels of MR-proADM compared with normal-weight, appropriate controls suggesting its important involvement in obese patients.

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Conflict of interest No conflict of interest.

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