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The Association of Pro-Atrial Natriuretic Peptide with Components of the Metabolic Syndrome in Healthy Adolescents and Young Adults from the General Population

Short title: proANP and Components of the Metabolic Syndrome

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ABSTRACT

BACKGROUND In middle-aged and elderly populations, circulating natriuretic peptide concentrations are negatively associated with several components of the metabolic syndrome. Whether these negative associations are also present in healthy adolescents and young adults from the general population are unknown.

METHODS In a cross-sectional setting, we measured plasma concentrations of mid-regional pro-atrial natriuretic peptide (MR-proANP) in 343 adolescents (age 14–16 years) and 616 young adults (age 20–28 years) from the Danish site of the European Youth Heart Study, which is a population-based study of cardiovascular disease risk factors in children, adolescents and young adults. We used linear regression analysis to examine the associations, expressed as standardized regression coefficients, of various variables of interest with MR-proANP stratified according to age group, adjusting for age and gender.

RESULTS Among the young adults, MR-proANP was negatively associated with body mass index (BMI) (β=-0.10, P=0.02), waist circumference (WC) (β=-0.14, P<0.001), systolic blood pressure (BP) (β=-0.08, P=0.03), diastolic BP (β=-0.23, P<0.001), insulin (β=-0.15, P<0.001), and triglycerides (β=-0.14, P<0.001). Among the adolescents a somehow different pattern was observed since MR-proANP was not significantly associated with BMI (β=-0.00, P=0.98), WC (β=-0.01, P=0.90) and insulin (β=-0.02, P=0.69). Nevertheless, among the adolescents, MR-proANP was negatively associated with triglycerides (β=-0.13, P=0.01), diastolic BP (β=-0.12, P=0.01) and systolic BP (β=-0.10, P=0.10), although the latter association was of borderline significance.

CONCLUSIONS The young adults displayed significant negative associations between MR-proANP and several components of the metabolic syndrome, whereas such associations were not found among the adolescents besides triglycerides and diastolic BP.

INTRODUCTION

The cardiac natriuretic peptides (NPs), atrial NP (ANP) and B-type NP (BNP), have diuretic, natriuretic and vasodilatory properties, and are thought to play a major role in blood pressure (BP) regulation.[1,2] Overweight is a major determinant of hypertension.[3] During the last decade, it has become clear that overweight adults have lower than expected circulating NP concentrations, and therefore it has been proposed that the lower amount of circulating NPs, resulting in diminished diuresis, natriuresis and vasodilation, could be involved in the pathogenesis of overweight-related hypertension.[3,4] The mechanisms underlying the relative NP deficiency in overweight adults are not fully understood, but estimates of insulin resistance (IR) and components belonging to the metabolic syndrome, such as higher body mass index (BMI), larger waist circumference (WC) and hypertriglyceridemia, have been found to be associated with lower circulating NP concentrations.[3-9] In the same period, it has also become clear that the NPs are involved in the regulation of lipolysis and lipid oxidation, and therefore it has been proposed that there is an important cardio-metabolic crosstalk between the endocrine heart, adipose tissue and muscle, and that this cross-talk could be disrupted in overweight-related diseases.[3,4]

Little is known about circulating NP concentrations in relation to anthropometric and metabolic parameters as well as BP in healthy adolescents and young adults (<30 years old) from the general population, although such relationships have been widely studied in community-based middle-aged and elderly persons (>40 years old).[5-7,9] Therefore, we studied a large sample of adolescents and young adults from the Danish site of the European Youth Heart Study (EYHS), which is an international population-based study of cardiovascular disease (CVD) risk factors in children, adolescents and young adults.[10,11] The EYHS includes standardised BP measurements, anthropometric measurements, such as BMI and WC,

measurements of lipids, insulin and glucose, and a bio-bank, enabling us to measure plasma concentrations of mid-regional pro-atrial NP (MR-proANP), a stable proANP fragment. MR-proANP measurement essentially seems to match that of the related proBNP with one major difference: circulating MR-proANP concentrations are much higher than that of proBNP-related peptides in non-cardiac patients and thus allow for accurate and reliable information on decreased concentrations compared with reference individuals.[12]

METHODS

Study population

This study is a cross-sectional study using Danish EYHS data.[11] A detailed description of the EYHS protocol has been reported elsewhere.[10] In 2009–2010, random samples of 709 14–16 year-old healthy adolescents, 469 20–22 year-old healthy adults and 658 26–28 year-old healthy adults were invited to take part in the Danish part of the study. The participation rate was 59% (*n*=399) for the adolescents and 46% (*n*=650) for the young adults. Exclusion criteria were pregnancy (*n*=12), diabetes (*n*=3) and non-fasting (*n*=32), leaving 959 individuals for the study. In the case of missing data, these were handled with the pairwise deletion technique, which involves deleting a case when it is missing a variable required for a particular analysis, but including that case in analyses for which all required variables are present. Because of this, total number varies in the various models. In analyses assessing IR, expressed by the Homeostatic Model Assessment 2 for IR (HOMA2-IR),[13] we excluded, as prescribed, individuals with fasting glucose concentrations outside the range of 3.5–25.0 mmol/L and with fasting insulin concentrations outside the range of 20–400 pmol/L (*n*=3 glucose <3.5 mmol/L, *n*=0 glucose >25.0 mmol/L, *n*=83 insulin <20 pmol/L and *n*=2 insulin > 400 pmol/L), leaving 873 individuals for the IR analyses. We performed age-group stratified

analyses because this would enable us to identify changes in the strength of the various associations with increasing age. The study was approved by the Regional Scientific Ethical Committee for Southern Denmark, and data were collected according to the Declaration of Helsinki. All participants gave their written informed consent.

Anthropometry and maturity

Weight, height, and WC were measured while the participants were wearing light clothing, without shoes. WC was measured midway between the lower rib margin and the iliac crest at the end of gentle expiration. BMI was calculated as weight (kg)/height^2 (m^2). Pubertal status was assessed according to Tanner,[14] as described elsewhere.[15] Maturity stage among the adolescents was almost exclusively stage 3, 4 and 5, thus, we collapsed maturity to a 3-point ordinal variable (Tanner 2-3, Tanner 4 and Tanner 5).

Blood measurements

Venous blood samples were drawn in the morning after at least 8 hours of fasting. Plasma concentrations of MR-proANP were measured with a commercially available sandwich chemiluminescence immunoassay (Thermo-Fisher, MA, USA) with intra-assay and inter-assay coefficients of variation below 2% and 6%, respectively, and a lower detection limit of 4.5 pmol/L. Glucose, high-density lipoprotein (HDL), total cholesterol, low-density lipoprotein (LDL) cholesterol and triglyceride concentrations were measured by standard methods using a Olympus AU600 random access analyser. Insulin was analysed using an enzyme immunoassay (microtiter plate format - Dako Diagnostics, Ely, U.K.).

BP measurements

BP was measured with participants in the upright sitting position after resting for 5 min, using a Dinamap monitor (Dinamap model XL- Kivex/Critikron, Tampa, FL, USA). Five measurements were conducted with 2 min intervals between each, and the mean of the last three measurements was used. The Dinamap monitor has previously been validated in children and adolescents against direct radial artery readings (mean error 0.24 mmHg systolic (S) BP and 1.28 mmHg diastolic (D) BP).[16]

Statistical analysis

All statistical analyses were performed in STATA 13.0 (STATA Corporation, College Station, TX, US). Normally distributed continuous data are presented as mean±standard deviation (SD) and non-normally distributed continuous data as median (interquartile range). Categorical data are presented as frequency percent. Group comparisons were performed using t-test for continuous data and chi-squared test for categorical data. To fulfil the model assumptions of the t-test, non-normally distributed variables (MR-proANP, triglycerides, insulin and HOMA2-IR) had to be log-transformed prior to group comparisons. Linear regression was used to examine the associations, expressed as standardized regression coefficients, of various variables of interest with MR-proANP stratified according to age group, adjusting for age and gender. In the regression models, non-normally distributed variables (MR-proANP, triglycerides, insulin and HOMA2-IR) had to be log-transformed to fulfil the model assumptions of linear regression. Furthermore, we specifically looked for age-group dependent MR-proANP interactions All statistical tests were 2-sided, and a *P*-value <0.05 was considered significant.

RESULTS

General characteristics of the study population are presented in Table 1. A few of these results have been published previously in a paper reporting the effect of gender and pubertal stage on circulating MR-proANP concentrations in adolescents.[15] As expected, our study subjects had overall normal BP and lipids.

Among the adolescents, males were significantly older, heavier and taller compared with females (P<0.01), but there were no significant gender differences in BMI and WC (P>0.09). Males had higher SBP but lower DBP compared with females, and they had lower LDL cholesterol (P<0.01). Regarding metabolic variables, males had higher glucose concentrations, but, despite higher SBP, they had lower plasma concentrations of MR-proANP (36.4 pmol/L (30.4–44.7) vs. 42.2 pmol/L (32.0–50.2), P<0.01), whereas there were no significant gender differences in insulin concentrations and HOMA2-IR (P>0.13).

In the adult group, males were taller, heavier, had higher BMI and larger WC compared with females (P<0.01). They had higher SBP and DBP, and they had lower HDL cholesterol (P<0.01). Concerning metabolic variables, males had higher glucose concentrations (P<0.01) but lower insulin concentrations (P<0.01) and lower degree of IR (P<0.05), and, despite higher SBP, they had lower plasma concentrations of MR-proANP (41.5 pmol/L (32.9–53.0) vs. 52.5 pmol/L (43.1–67.7), P<0.01).

With respect to gender differences between age groups, adult females were heavier, had higher BMI and larger WC compared with adolescent females (P<0.05). They also had higher DBP, but not higher SBP, and they had higher lipid concentrations (P<0.05). Regarding metabolic variables, the adult females had higher glucose concentrations and higher plasma concentrations of MR-proANP but lower insulin concentrations and lower degree of IR compared with adolescent females (P<0.05). Regarding differences between adolescent males and adult males, adult males were taller, heavier, had higher BMI and larger WC (P<0.05).

They had higher BP and higher triglyceride and LDL cholesterol concentrations ($p<0.05$). Regard metabolic variables, adult males had higher glucose concentrations and higher plasma concentrations of MR-proANP, but lower insulin concentrations and degree of IR ($P<0.05$).

Table 2 summarizes linear regression-based associations of plasma MR-proANP with variables of interest. Among the adolescents, all anthropometric, hemodynamic, lipid (except for HDL cholesterol) and metabolic variables were negatively associated with MR-proABP, but the strength of the associations only reached statistical significance regarding triglycerides and DBP ($P<0.04$). Nevertheless, the strength of the association between SBP and MR-proANP was borderline significant ($P=0.10$). Among the adults, all anthropometric, hemodynamic, lipid (except for HDL cholesterol) and metabolic variables were negatively associated with MR-proANP, and the strength of the associations reached statistical significance for all variables except for glucose ($P=0.16$). Numerically, 9 of the 10 variables showed stronger associations among the adults compared with the adolescents, and for BMI, WC, DBP and HDL-cholesterol, the strength of the associations were statistically significant different between the age groups, as reflected by a $P<0.05$ in the interaction analyses.

Finally, associations of SBP and DBP with variables of interest are displayed in Table 3 and 4 to document that the expected associations between anthropometric and metabolic variables and BP were also found in our study population.

Sensitivity analysis

Adjusting for Tanner stage did not materially change any of the relationships with MR-proANP among the adolescents (data not shown).

DISCUSSION

This study is the first to have measured plasma MR-proANP in a large sample of adolescents and young adults from the general population. Among the adolescents, the well-described negative relationships between the NPs and BMI, WC, insulin and estimates of IR seen later in life were not present.[3-9] In contrast, among the young adults, all these well-described relationships were present. The only significant metabolic relationship found among the adolescents was a negative relationship between plasma MR-proANP and triglycerides. This relationship was also present in the young adults in whom plasma MR-proANP was also positively associated with HDL-cholesterol. Furthermore, we found that in both study groups plasma MR-proANP were negatively associated with DBP and in the adult study group also with SBP. Finally, based on our interaction analyses, the strength of the relationships between MR-proANP and BMI, WC and DBP were significantly stronger among the young adults compared with the adolescents. Thus our study, although cross-sectional, may provide some understanding about changes in circulating NP concentrations with changes in anthropometry and BP over time.

NPs in children and adolescents

Several smaller studies (n<150), almost all hospital-based,[17-23] have studied the relationships between NPs and anthropometry, metabolism and BP in children and adolescents. The studies are heterogeneous, and the results are conflicting. Most studies measured proBNP-related peptides which in non-cardiac patients circulate in much lower concentrations than that of proANP-related peptides, thus making it difficult to obtain accurate and reliable information on decreased concentrations compared with reference individuals.[12]

In a Greek study, from an obesity clinic, involving 47 girls and 49 boys, aged 10–16 years, without cardiac, hepatic and renal diseases, plasma NT-proBNP concentrations did not

correlate with BMI neither in girls nor in boys, although the obese boys, despite higher SBP (119.7±19.1 vs. 106.5±13.1 mm Hg, *P*<0.001), had non-significantly lower plasma NT-proBNP concentrations (31.4 8±14.3 pg/mL vs. 46.9 8±33.9 pg/mL) compared with the normal weight boys.[17] One could have expected that the higher SBP would have been associated with higher plasma NT-proBNP concentrations.[1] Furthermore, plasma NT-proBNP concentrations did not correlate with SBP and DBP neither in girls nor in boys, although among the obese boys plasma NT-proBNP concentrations correlated significantly with SBP (*r*=0.45, *P*=0.03).[17] In a second report from the same population,[18] focusing further on metabolic relationships, plasma NT-proBNP concentrations correlated negatively with HDL-cholesterol (*r*=-0.36, *P*=0.03) in boys, a finding in contrast with our findings, whereas no significant correlations were found between plasma NT-proBNP and glucose, insulin, HOMA index, cholesterol and triglycerides. In these studies, no further adjusted analyses or interaction analyses were reported.

In an Italian study, performed in a hospital setting, 29 obese adolescents without cardiac dysfunction, mean age 11.8±0.4 years, mean BMI 29.8 0.82 kg/m^2, were found to have lower plasma BNP concentrations (18.8±2.6 vs 36.9±5.5 pg/mL, *P*=0.003) compared with reference values specific for Italian children and adolescents.[19] In that study, no information regarding BNP and BP were provided, but among the 29 obese adolescents BNP was reported to correlated with glycaemia (*r*=0.33, *P*<0.05), HDL-cholesterol (*r*=0.4, *P*=0.029) and triglycerides (*r*=0.5, *P*=0.005). However, in another Italian study from the same group, also from a hospital setting, involving 82 children and adolescents, mean age 12.8±2.4 years, of whom 27 were normal weight, 10 overweight and 45 obese, no significant differences were found in circulating concentrations of NT-proBNP or MR-proANP among the 3 groups. This

result did not change after further adjustment for age and sex.[20] Also in this study, no BP data was provided.[20]

In a Turkish study, done in a hospital setting, including 50 obese children and 20 age- and sex-matched healthy normal weight control children, mean age around 10 years, the obese children had significantly higher plasma NT-proBNP concentrations (109.25±48.53 vs. 51.96±22.36 pg/mL, *P*=0.001) compared with the normal weight children.[21] In that study, plasma NT-proBNP concentrations were not associated with BP, although the obese children had higher SBP and DBP compared with the normal weight children.[21] In another Turkish study, also from a hospital setting, involving 68 obese children and 35 healthy controls matched for age and sex, no significant difference was found in plasma NT-proBNP concentrations (56.9±32.9 vs. 53±40.1 pg/mL, *P*=0.46) between the 2 groups, although the obese children had significantly higher SBP (120±15 vs. 106±9 mm Hg, *P*=0.001).[22] Furthermore, no differences were found in plasma NT-proBNP concentrations when the study population was subdivided according to presence of the metabolic syndrome, hypertension or IR.

In a Chinese study, involving 114 children and adolescents, 32% females, with a median age of 10.8 years, who were enrolled because of habitual snoring and symptoms suggestive of obstructive sleep apnoea, stepwise multiple regression analysis identified BMI z-score as the only factor significantly (and negatively) associated with ANP and BNP.[23] In this context, it is worth noting that the Chinese children and adolescents, who based on z-score were defined to be obese, had higher BP and lower plasma concentrations of ANP and BNP compared with the non-obese Chinese children and adolescents.[23]

Among 138 children and adolescents, age 5 to 17 years, 55% females, recruited as part of a population-based genetic study from 3 remote villages in southern Italy, BMI was not

associated with Nt-proBNP (r=0.003, P=0.97) in univariate analysis, whereas WC was (r=-0.30, P<0.001).[24] In that study, no BP measurements were reported, and after adjustment for age, only age was associated with Nt-proBNP.[24]

NPs and BP

The relationship between circulating NP concentrations and BP is complex and non-linear. It is well-established that hypertensive patients with severe hypertension and signs of hypertensive heart disease with enlargement of the left ventricle or the left atrium have increased circulating concentrations of NPs.[25-30] However, several studies,[31] including genetic studies,[32,33] have shown that NPs in the high normal range are associated with lower BP and lower risk of hypertension. Our data confirms this observation. Thus, in our healthy adolescents and young adults from the general population, higher circulating MR-proANP concentrations were associated with lower BP. This suggests that a low amount of circulating NPs could play a role in the early stages of hypertension development.[34-36]

NP and age

In this study, the young adults had higher circulating MR-proANP concentrations compared with the adolescents. In this context it is worthy of note that a study from the Framingham Heart study, consisting of a healthy reference sample of 911 subjects (mean age 55 years, 62% women) who were free of hypertension, valvular disease, diabetes, atrial fibrillation, obesity, coronary heart disease, congestive heart failure, and renal failure, and who had normal left ventricular systolic function, showed that the strongest predictors of higher natriuretic peptide levels were older age and female sex.[37] Why our young adults had higher circulating MR-proANP concentrations is not exactly clear, because between the 2 age groups,

significant differences were found both in factors expected to be associated with lower and higher circulating NP concentrations, respectively Thus, the young adults had higher BMI, larger WC and hypertriglyceridemia, factors expected to be associated with lower circulating NP concentrations, but they also had lower insulin levels and lower degree of IR, elements expected to be associated with higher circulating NP concentrations. Clearly more research is needed in this area.

Strengths and limitations

The strengths of this study are its large sample size (although the participation rate was modest), its community-based nature and its measurement of a proANP-related peptide, because in the normal heart, it is predominantly ANP not BNP that is released in response to normal physiological stimuli.[12] It is a limitation of this study that we have no measurements of factors, for example sodium intake and body composition, that could have affected our MR-proANP results.[1,2,38,39] It is also a limitation that we have no follow-up data, because in a prospective population-based study, higher circulating concentrations of MR-proANP was associated with lower prevalence of IR at follow-up 16.5 years later.[40]

Conclusions

In this study, among healthy young adults from the general population, we found basically the same relationships between a NP-related peptide and anthropometry, metabolism and BP as found in several other studies including community-based middle-aged and elderly persons.[3-9,37-39]. However, among healthy adolescents from the general population, we only found relationships between a NR-related peptide and triglycerides and DBP. Nevertheless, our results do support the view that lower circulating concentrations of NPs could play a role

in the early stages of hypertension development. Future prospective studies are needed to track changes in circulating NP concentrations with changes in anthropometry, metabolism and BP from childhood to adolescents and from adolescents to adulthood.

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DISCLOSURE

The authors declared no conflicts of interests.

REFERENCES

1. Levin ER, Gardner DG, Samson WK. Natriuretic peptides. N Engl J Med. 1998 ;339:321-328.
2. Volpe M, Carnovali M, Mastromarino V. The natriuretic peptides system in the pathophysiology of heart failure: from molecular basis to treatment. Clin Sci (Lond). 2016;130:57-77.
3. Jordan J, Birkenfeld AL. Cardiometabolic crosstalk in obesity-associated arterial hypertension. Rev Endocr Metab Disord. 2016;17:19-28.
4. Coué M, Moro C. Natriuretic peptide control of energy balance and glucose homeostasis. Biochimie. 2016;124:84-91.
5. Olsen MH, Hansen TW, Christensen MK, Gustafsson F, Rasmussen S, Wachtell K, Borch-Johnsen K, Ibsen H, Jørgensen T, Hildebrandt P. N-terminal pro brain natriuretic

peptide is inversely related to metabolic cardiovascular risk factors and the metabolic syndrome. Hypertension. 2005;46:660-666

6. Wang TJ, Larson MG, Keyes MJ, Levy D, Benjamin EJ, Vasan RS. Association of plasma natriuretic peptide levels with metabolic risk factors in ambulatory individuals. Circulation. 2007;115:1345-1353.
7. Khan AM, Cheng S, Magnusson M, Larson MG, Newton-Cheh C, McCabe EL, Coviello AD, Florez JC, Fox CS, Levy D, Robins SJ, Arora P, Bhasin S, Lam CS, Vasan RS, Melander O, Wang TJ. Cardiac natriuretic peptides, obesity, and insulin resistance: evidence from two community-based studies. J Clin Endocrinol Metab. 2011;96:3242-3249.
8. Asferg CL, Nielsen SJ, Andersen UB, Linneberg A, Møller DV, Hedley PL, Christiansen M, Gøtze JP, Jeppesen JL. Metabolic rather than body composition measurements are associated with lower serum natriuretic peptide concentrations in normal weight and obese men. Am J Hypertens. 2014;27:620-627.
9. Jujić A, Nilsson PM, Persson M, Holst JJ, Torekov SS, Lyssenko V, Groop L, Melander O, Magnusson M. Atrial Natriuretic Peptide in the High Normal Range Is Associated With Lower Prevalence of Insulin Resistance. J Clin Endocrinol Metab. 2016;101:1372-1380.
10. Riddoch C,Edwards D, Page A, Froberg K, Anderssen SA, Wedderkopp N, Brage S, Cooper AR, Sardinha LB, Harro M, Klasson-Heggebø L, van Mechelen W, Boreham C, Ekelund U, Andersen LB and the European Youth Heart Study team. The European Youth Heart Study–cardiovascular disease risk factors in children: rationale, aims, design and validation of methods. J Phys Act Health. 2005;2:115–129.
11. Ried-Larsen M, Grøntved A, Østergaard L, Cooper AR, Froberg K, Andersen LB, Møller NC. Associations between bicycling and carotid arterial stiffness in adolescents: The European Youth Hearts Study. Scand J Med Sci Sports. 2015;25:661-669.

12. Goetze JP, Hansen LH, Terzic D, Zois NE, Albrethsen J, Timm A, Smith J, Soltysinska E, Lippert SK, Hunter I. Atrial natriuretic peptides in plasma. Clin Chim Acta. 2015;443:25-28.
13. Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. Diabetes Care. 1998;21:2191-2192.
14. Tanner JM. Growth and maturation during adolescence. Nutr Rev. 1981;39:43–55.
15. Goharian TS, Gimsing AN, Goetze JP, Faber J, Andersen LB, Grøntved A, Jeppesen JL. Mid-regional pro-atrial natriuretic peptide and blood pressure in adolescents: effect of gender and pubertal stage. Blood Press. 2015;24:347-352.
16. Park MK, Menard SM. Accuracy of blood pressure measurement by the Dinamap Monitor in infants and children. Pediatrics. 1987;79:907–914.
17. Pervanidou P, Akalestos A, Sakka S, Kanaka-Gantenbein C, Papassotiriou I, Chrousos GP. Gender dimorphic associations between N-terminal pro-brain natriuretic peptide, body mass index and blood pressure in children and adolescents. Horm Res Paediatr. 2010;73:341-348.
18. Pervanidou P, Margeli A, Akalestos A, Sakka S, Kanaka-Gantenbein C, Papassotiriou I, Chrousos GP. Associations between circulating N-terminal pro-Brain Natriuretic Peptide (NT-proBNP) and adiponectin concentrations depend on obesity level in female adolescents: gender dimorphic findings. Horm Metab Res. 2009;41:829-833.
19. Del Ry S, Cabiati M, Bianchi V, Storti S, Caselli C, Prescimone T, Clerico A, Saggese G, Giannessi D, Federico G. C-type natriuretic peptide plasma levels are reduced in obese adolescents. Peptides. 2013;50:50-54.
20. Del Ry S, Cabiati M, Bianchi V, Caponi L, Maltinti M, Caselli C, Kozakova M, Palombo C, Morizzo C, Marchetti S, Randazzo E, Clerico A, Federico G. C-type natriuretic peptide is

closely associated to obesity in Caucasian adolescents. Clin Chim Acta. 2016;460:172-177.

21. Saritas T, Tascilar E, Abaci A, Yozgat Y, Dogan M, Dundaroz R, Hasimi A, Yesilkaya E, Lenk MK, Kilic A. Importance of plasma N-terminal pro B-type natriuretic peptide, epicardial adipose tissue, and carotid intima-media thicknesses in asymptomatic obese children. Pediatr Cardiol. 2010;31:792-799.
22. Battal F, Ermis B, Aktop Z, Can M, Demirel F. Early cardiac abnormalities and serum N-terminal pro B-type natriuretic peptide levels in obese children. J Pediatr Endocrinol Metab. 2011;24:723-726.
23. Li AM, Au CT, Zhu JY, Chan KC, Chan MH, Lee DL, Wing YK. Plasma natriuretic peptides in children and adolescents with obstructive sleep apnea and their changes following intervention. Front Pediatr. 2014 Mar 24;2:22. doi: 10.3389/fped.2014.00022. eCollection 2014.
24. Siervo M, Ruggiero D, Sorice R, Nutile T, Aversano M, Iafusco M, Vetrano F, Wells JC, Stephan BC, Ciullo M. Body mass index is directly associated with biomarkers of angiogenesis and inflammation in children and adolescents. Nutrition. 2012;28:262-266.
25. Wambach G, Bönner G, Stimpel M, Kaufmann W. Relationship between plasma atrial natriuretic peptide and left atrial and left ventricular involvement in essential hypertension. J Hypertens 1988;6:573-577.
26. Nadir MA, Rekhraj S, Wei L, Lim TK, Davidson J, MacDonald TM, Lang CC, Dow E, Struthers AD. Improving the primary prevention of cardiovascular events by using biomarkers to identify individuals with silent heart disease. J Am Coll Cardiol 2012;60:960-968.

27. Ganau A, Devereux RB, Atlas SA, Pecker M, Roman MJ, Vargiu P, Cody RJ, Laragh JH. Plasma atrial natriuretic factor in essential hypertension: relation to cardiac size, function and systemic hemodynamics. J Am Coll Cardiol 1989;14:715-724.
28. Sagnella GA, Markandu ND, Shore AC, MacGregor GA. Raised circulating levels of atrial natriuretic peptides in essential hypertension. Lancet 1986;1:179-181.
29. Montorsi P, Tonolo G, Polonia J, Hepburn D, Richards AM. Correlates of plasma atrial natriuretic factor in health and hypertension. Hypertension 1987;10:570-576.
30. Kohno M, Yasunari K, Matsuura T, Murakawa K, Takeda T. Circulating atrial natriuretic polypeptide in essential hypertension. Am Heart J 1987;113:1160-1163.
31. Xanthakis V, Enserro DM, Murabito JM, Polak JF, Wollert KC, Januzzi JL, Wang TJ, Tofler G, Vasan RS. Ideal cardiovascular health: associations with biomarkers and subclinical disease and impact on incidence of cardiovascular disease in the Framingham Offspring Study. Circulation. 2014;130:1676-1683.
32. Newton-Cheh C, Larson MG, Vasan RS, Levy D, Bloch KD, Surti A, Guiducci C, Kathiresan S, Benjamin EJ, Struck J, Morgenthaler NG, Bergmann A, Blankenberg S, Kee F, Nilsson P, Yin X, Peltonen L, Vartiainen E, Salomaa V, Hirschhorn JN, Melander O, Wang TJ. Association of common variants in NPPA and NPPB with circulating natriuretic peptides and blood pressure. Nat Genet 2009;41:348-53.
33. Jeppesen J, Nielsen SJ, Torp-Pedersen C, Hansen TW, Olsen MH, Berg ND, Linneberg A, Madsbad S, Femger M. Genetic variation in the natriuretic peptide system, circulating natriuretic peptide levels, and blood pressure: an ambulatory blood pressure study. Am J Hypertens 2012; 25:1095-1100.
34. Dessì-Fulgheri P, Sarzani R, Tamburrini P, Moraca A, Espinosa E, Cola G, Giantomassi L, Rappelli A. Plasma atrial natriuretic peptide and natriuretic peptide receptor gene

expression in adipose tissue of normotensive and hypertensive obese patients. J Hypertens 1997;15:1695-1699.

35. Asferg CL, Nielsen SJ, Andersen UB, Linneberg A, Møller DV, Hedley PL, Christiansen M, Goetze JP, Esler M, Jeppesen JL. Relative atrial natriuretic peptide deficiency and inadequate renin and angiotensin II suppression in obese hypertensive men. Hypertension 2013;62:147-153.
36. Seven E, Husemoen LL, Ibsen H, Friedrich N, Nauck M, Wachtell K, Linneberg A, Jeppesen JL. Higher serum concentrations of N-terminal pro-B-type natriuretic peptide associate with prevalent hypertension whereas lower associate with incident hypertension. PLoS One 2015 Feb 6;10(2):e0117864. doi10.1371/journal.pone.0117864. eCollection 2015.
37. Wang TJ, Larson MG, Levy D, Leip EP, Benjamin EJ, Wilson PW, Sutherland P, Omland T, Vasan RS. Impact of age and sex on plasma natriuretic peptide levels in healthy adults. Am J Cardiol. 2002;90:254-258.
38. Neeland IJ, Winders BR, Ayers CR, Das SR, Chang AY, Berry JD, Khera A, McGuire DK, Vega GL, de Lemos JA, Turer AT. Higher natriuretic peptide levels associate with a favorable adipose tissue distribution profile. J Am Coll Cardiol. 2013;62:752-760.
39. Cheng S, Fox CS, Larson MG, Massaro JM, McCabe EL, Khan AM, Levy D, Hoffmann U, O'Donnell CJ, Miller KK, Newton-Cheh C, Coviello AD, Bhasin S, Vasan RS, Wang TJ. Relation of visceral adiposity to circulating natriuretic peptides in ambulatory individuals. Am J Cardiol. 2011;108:979-984.
40. Jujić A, Nilsson PM, Persson M, Holst JJ, Torekov SS, Lyssenko V, Groop L, Melander O, Magnusson M. Atrial Natriuretic Peptide in the High Normal Range Is Associated With Lower Prevalence of Insulin Resistance. J Clin Endocrinol Metab. 2016;101:1372-1380.

Table 1. General characteristics of the participants stratified by age group and gender

Variables	Adolescents, *n*=335–343		Young Adults, *n*=611–616	
	Female, *n*=187–191	Male, *n*=148–152	Female, *n*=319–322	Male, *n*=292–294
Demographic				
Age, years	15.6±0.36	15.7±0.41*	24.1±3.0†	24.4±3.0†
Occasional or regular smoking (%)	28.2%	24.7%	22.8%	32.4%*
Frequency of alcohol intake, once/month or more often (%)	64.4%	63.2%	70.9	87.0*
Anthropometric				
Height, cm	166.5±6.5	177.0±7.1*	167.1±6.7	180.9±6.8*†
Weight, kg	58.8±9.5	65.0±10.7*	66.1±12.2†	81.4±14.4*†
BMI, kg/m2	21.2±3.2	20.7±3.1	23.7±4.3†	24.8±3.9*†
Waist circumference, cm	71.9±7.3	73.3±8.7	77.1±9.8†	84.5±10.7*†
Hemodynamic				
Systolic BP, mmHg	109.6±9.9	116.8±11.4*	110.5±8.7	120.0 9.8*†
Diastolic BP, mmHg	62.9±6.3	60.4±6.3*	68.9±7.6†	70.6±8.3*†
Lipid				
Triglycerides, mmol/L	0.9 (0.6–1.3)	0.8 (0.6–1.1)	1.1 (0.8-1.4)†	1.1 (0.8–1.4)†
HDL cholesterol, mmol/L	1.3±0.3	1.2±0.3	1.5±0.3†	1.2±0.3*
LDL cholesterol, mmol/L	2.4±0.6	2.2±0.6*	2.7±0.8†	2.7±0.7†
Metabolic and cardiac				
Glucose, mmol/L	4.8±0.5	5.0±0.4*	4.9±0.4†	5.2±0.4*†
Insulin, pmol/L	50.4 (37.1–66.3)	46.5 (34.5–61.5)	44.0 (32.9–61.1)†	33.7 (23.9–50.1)*†
HOMA2-IR, units	1.5 (1.1–2.2)	1.5 (1.2–2.0)	1.4 (1.1–2.0)†	1.3 (1.0–1.8)†‡
MR-proANP, pmol/L	42.2 (32.0–50.2)	36.4 (30.4–44.7)*	52.5 (43.1–67.7)†	41.5 (32.9–53.0)*†

Tanner				
Post pubertal[a], (%)	85	88	-	-

Data are presented as mean ±SD or median (interquartile range) or percent frequency. BMI: body mass index, BP: blood pressure, HDL: high-density lipoprotein, LDL: low-density lipoprotein, HOMA2-IR: homeostasis model assessment of insulin resistance, MR-proANP: mid-regional pro-atrial natriuretic peptide. [a]Tanner stage 4 and 5. [*]Difference between gender $P<0.01$. [†]Difference within gender between age group $P<0.05$. [‡]Difference between gender $P<0.05$.

Table 2. Linear regression-based associations of serum MR-proANP with variables of interest stratified by age group

Variables	Adolescents, *n*=335–343		Young Adults, *n*=611–616		
	Standardized beta or odds ratio (95% CI)	*P*	Standardized beta or odds ratio (95% CI)	*P*	*P* for age-group by MR-proANP interaction
BMI, kg/m2	-0.001 (-0.09–0.09)	0.98	-0.10 (-0.19– -0.02)	0.02	0.04
Waist circumference, cm	-0.01 (-0.09–0.08)	0.90	-0.14 (-0.22– -0.06)	<0.001	0.003
Systolic BP, mmHg	-0.10 (-0.21–0.02)	0.10	-0.08 (-0.16– -0.01)	0.03	0.92
Diastolic BP, mmHg	-0.12 (-0.21– -0.03)	0.01	-0.23 (-0.31– -0.15)	<0.001	0.01
Triglycerides, mmol/L	-0.13 (-0.24– -0.01)	0.03	-0.14 (-0.22– -0.06)	<0.001	0.82
HDL cholesterol, mmol/L	0.03 (-0.08–0.14)	0.57	0.17 (0.09– 0.25)	<0.001	0.002
LDL cholesterol, mmol/L	-0.08 (-0.17–0.02)	0.14	-0.11 (-0.20– -0.02)	0.01	0.50
Glucose, mmol/L	-0.04 (-0.16–0.08)	0.50	-0.06 (-0.14–0.02)	0.16	0.49
Insulin, pmol/L	-0.02 (-0.13–0.09)	0.69	-0.15 (-0.24– -0.07)	<0.001	0.24
HOMA2-IR, units	-0.03 (-0.14–0.07)	0.56	-0.10 (-0.18– -0.03	0.01	0.26

All data are and adjusted for age and gender. BMI: body mass index, BP: blood pressure, HDL: high-density lipoprotein, LDL: low-density lipoprotein, HOMA2-IR: homeostasis model assessment of insulin resistance, MR-proANP: mid-regional pro-atrial natriuretic peptide.

Table 3. Linear regression-based associations of variables of interest with systolic blood pressure stratified by age group

Variables	Adolescents, *n*=335–343		Young Adults, *n*=611–616		
	Standardized beta (95% CI)	*P*	Standardized beta (95% CI)	*P*	*P* for age-group by variable of interest interaction
BMI, kg/m2	0.43 (0.30–0.56)	<0.001	0.27 (0.21–0.34)	<0.001	0.02
Waist circumference, cm	0.43 (0.29–0.56)	<0.001	0.27 (0.20–0.34)	<0.001	0.03
Triglycerides, mmol/L	0.16 (0.05–0.26)	0.005	0.24 (0.17–0.31)	<0.001	0.23
HDL cholesterol, mmol/L	-0.03 (-0.14–0.09)	0.64	-0.03 (-0.10–0.04)	0.45	0.72
LDL cholesterol, mmol/L	0.20 (0.07–0.32)	0.002	0.10 (0.03–0.16)	0.005	0.08
Glucose, mmol/L	0.07 (-0.04–0.18)	0.20	0.17 (0.10–0.24)	<0.001	0.03
Insulin, pmol/L	0.16 (0.05–0.27)	0.004	0.24 (0.18–0.31)	<0.001	0.29
HOMA2-IR, units	0.16 (0.04–0.28)	0.01	0.25 (0.17–0.33)	<0.001	0.18
MR-proANP	-0.10 (-0.21–0.02)	0.10	-0.08 (-0.16– -0.01)	0.03	0.92

All data are and adjusted for age and gender. BMI: body mass index, BP: blood pressure, HDL: high-density lipoprotein, LDL: low-density lipoprotein, HOMA2-IR: homeostasis model assessment of insulin resistance, MR-proANP: mid-regional pro-atrial natriuretic peptide.

Table 4. Linear regression-based associations of diastolic blood pressure with variables of interest stratified by age group

Variables	Adolescents, *n*=335–343		Young Adults, *n*=611–616		
	Standardized beta (95% CI)	*P*	Standardized beta (95% CI)	*P*	*P* for age-group by variable of interest interaction
BMI, kg/m2	0.14 (0.04–0.25)	0.007	0.18 (0.10–0.25)	<0.001	0.72
Waist circumference, cm	0.14 (0.03–0.25)	0.012	0.23 (0.15–0.30)	<0.001	0.09
Triglycerides, mmol/L	0.11 (0.03–0.19)	0.009	0.34 (0.27–0.41)	<0.001	<0.001
HDL cholesterol, mmol/L	-0.04 (-0.13–0.05)	0.40	-0.08 (-0.15–0.002)	0.056	0.17
LDL cholesterol, mmol/L	0.09 (-0.002–0.19)	0.055	0.10 (0.03–0.17)	0.008	0.60
Glucose, mmol/L	0.10 (0.02–0.18)	0.017	0.23 (0.15–0.30)	<0.001	0.001
Insulin, pmol/L	0.16 (0.07–0.25)	<0.001	0.28 (0.21–0.36)	<0.001	0.15
HOMA2-IR, units	0.16 (0.06–0.25)	0.001	0.31 (0.22–0.40)	<0.001	0.03
MR-proANP	-0.12 (-0.21– -0.03)	0.010	-0.23 (-0.31– -0.15)	<0.001	0.01

All data are and adjusted for age and gender. BMI: body mass index, BP: blood pressure, HDL: high-density lipoprotein, LDL: low-density lipoprotein, HOMA2-IR: homeostasis model assessment of insulin resistance, MR-proANP: mid-regional pro-atrial natriuretic peptide.