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Atrial Natriuretic Peptide in the high normal range is associated with lower prevalence of insulin resistance

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Abstract

Context We have previously shown that high levels of atrial natriuretic peptides (ANP) are associated with decreased risk of future diabetes development; however, the mechanism behind this relationship is not fully understood.

Objective In this study, we prospectively analyzed whether baseline plasma levels of mid-regional proANP (MR-proANP) are associated with insulin resistance and post challenge incretin secretion after long-term follow-up.

Design/Setting/Patients MR-proANP was measured in 2243 non-diabetic individuals at baseline examination of Malmö Diet and Cancer Cardiovascular cohort. At re-examination 16.5 years later, glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1), insulin, glucose and glucagon were measured during an oral glucose tolerance test.

Results Linear regression analyses showed that each 1 SD increment of baseline MR-proANP levels was inversely associated with insulin resistance calculated as HOMA-IR (per 1 SD change β= -0.066, p-value 0.001) at follow-up. Logistic regression analysis showed that each 1 SD increment of baseline ANP levels resulted in lower risk of belonging to upper quartile of HOMA-IR at follow-up (OR 0.88; CI 95% 0.78-0.99; p-value 0.043). In linear regression analyses each 1 SD increment in baseline MR-proANP levels was associated with greater GIP release (per 1 SD change: β=0.055; p-value=0.020) 120 minutes after 75g glucose intake, but not with GLP-1 release (per 1 SD change: β=0.016; p-value=0.493) 120 minutes after 75g glucose intake at 16.5 years of follow up.

Conclusion Midlife exposure to ANP within the high normal range is associated with lower risk of insulin resistance. Further, midlife exposure to ANP within the high normal range is associated with greater post challenge GIP secretion at follow-up, possibly explaining the lower prevalence of insulin resistance.

Key words: atrial natriuretic peptide, glucagon, glucose, glucose-dependent insulinotropic peptide, glucagon-like peptide-1, insulin, insulin resistance

Introduction

Natriuretic peptides (NPs) are potent cardiac hormones that play a key role in blood pressure control and cardiac remodeling. Atrial natriuretic peptide (ANP) is predominantly secreted by the cardiac atria, whilst brain natriuretic peptide (BNP) is predominantly secreted by cardiac ventricles. Cardiac myocytes produce ANP as a preprohormone that is stored as proANP. The enzyme corin processes the precursor proANP into biologically active, mature ANP and its inactive fragment N-terminal proANP. (1,2)

Recent studies demonstrated that NPs also play an important role in metabolic processes and control of energy usage. Both ANP and BNP have been shown to have lipolytic properties (3) resulting in a fatty acid release which in turn is associated with increased insulin concentrations. (4) Cross-sectional studies reveal that both NPs are reduced in subjects with obesity, insulin resistance and type 2 diabetes (T2D) (5) and low levels of both NPs have been shown to predict future T2D. (6,7) Furthermore, a common genetic variant, rs5068, earlier shown to be genome wide significantly associated with higher levels of ANP, is also associated with lower prevalence of new onset T2D, proposing a causal relationship between ANP and T2D-development. (8) However, the mechanisms behind these associations are unclear.

Another group of hormones also involved in T2D are incretins, a group of intestinal hormones that potentiate the glucose-dependent insulin response following nutrient intake in humans and has subsequent blood glucose lowering effects. Most of the incretin-effect is accounted for by glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1). Both GIP and GLP-1 stimulate glucose dependent insulin secretion when binding to their receptors, GIP receptor (GIPR) and GLP-1 receptor (GLP-1R), which results in activation of adenylate cyclase and increased levels of cAMP in pancreatic β-cells. However, GIPR and GLP-1R are also expressed in a broad spectrum of tissue including the heart and coronary artery endothelial cells and are rapidly degraded by dipeptidyl peptidase-4 enzyme (DDP-4). Further, GIP plays an important role in lipid metabolism by activation

of lipoprotein lipase (9) and GLP-1 has been demonstrated to have receptor-independent cardio-protective effects in GLP-1R knock out mice. (10)

The incretin system seems to be impaired in patients with T2D. (11) Treatment with GLP-1 analogs and DDP-4 inhibitors has shown promising effects on glucose control, but there is also evidence that treatment with DPP-4 inhibitors and GLP-1 analogs lower blood pressure, possibly because GLP-1 induces natriuresis. (12) Further, a recent study in mice pointed to the existence of a "gut-heart" GLP-1R-dependent and ANP-dependent axis that regulates blood pressure through promotion of ANP secretion and subsequent reduction of blood pressure. (13) Nevertheless, Skov et al. found no evidence of GLP-1 and ANP associations in 12 healthy human subjects. (14) In this prospective, observational study, we hypothesized that midlife exposure to ANP levels in the high normal range is protective of insulin resistance at follow up 16 years later. Further, we hypothesized that midlife exposure to ANP in the high normal range might affect post challenge incretin, glucose, insulin and glucagon levels after 16.5 years of follow-up in a large non-diabetic population-based study cohort.

Methods

Ethics statement

The study was approved by the Ethical Review Board at Lund University. A written informed consent was obtained from all subjects.

Subjects

Between 1991 and 1996, baseline examinations including anthropometrical measurements and blood sample donations were performed within The Malmo Diet and Cancer Study (MDC), a prospective population-based study (n= 30,447) in the city of Malmo, Sweden. In order to study cardiovascular risk factors, a subsample of the study population (n=6103) was randomized into a sub study The Malmö Diet and Cancer Cardiovascular Cohort (MDC-CC). Self-reported physician diagnosis of T2D or use of diabetes medication or fasting whole blood glucose of >6.1 mmol/L was considered as evidence for diabetes. During 2007-2012, a new clinical re-examination was performed within the

MDC-CC cohort with addition of oral glucose tolerance test (OGTT) in 3734 subjects. After excluding patients with diabetes at baseline (n=267) and incident diabetes at follow-up (n=486), complete data on all covariates were available in 2243 subjects. A complete description of the study population has been given elsewhere. (15)

Clinical assessment

Baseline examination included anthropological measurements and blood samples drawn after overnight fast. Antihypertensive treatment (AHT) was defined as use of beta-receptor blockers or angiotensin-converting-enzyme (ACE) inhibitors, or calcium antagonists, or diuretics. Blood pressure was obtained after 10 min of rest in the supine position. Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters.

Laboratory assays

Baseline fasting blood glucose (FBG) was analyzed at the time of the baseline examination (1991-1996) at the Department of Clinical Chemistry, Skåne University Hospital in Malmö, which is part of a national standardization and quality control system. Additional blood samples were stored at -80°C from baseline collection until 2007, when mid-regional proANP (MR-proANP) was measured using an immunoluminometric sandwich assay targeted against amino acids in the mid-region of the peptide (BRAHMS AG, Hennigsdorf, Germany), with mean interassay coefficients of variation ≤ 10%. The lower detection limit, determined with horse serum, was 6.0 pmol/L. Complete description of the MR-proANP assay has been published elsewhere. (2)

At re-examination, a protocol similar to the baseline protocol was applied, with addition of OGTT. During OGTT, blood samples were drawn in order to analyze GIP and GLP-1 at 0 and 120 minutes. Total plasma GLP-1 concentrations (intact GLP-1 and the metabolite GLP-1 9-36 amide) were determined radioimmunologically as described previously (minimum detection limit 1 pmol/L; intra- and inter-assay coefficients of variation <6.0 % and <15 %, respectively). (16) Identical quality controls and identical batches for all reagents in each analysis set were used in a consecutive sample analysis during two months. Serum GIP was analyzed using Millipore's Human GIP Total ELISA

#EZHGIP-54K (minimum detection level 1.65 pmol/L, intra- and inter-assay coefficients of variation were 1.8–6.1%, and 3–8.8% respectively). Fasting plasma glucose (FPG) was analyzed after an overnight fast using the Hemocue Glucose System (HemoCue AB, Ängelholm, Sweden). Serum insulin was assayed with Dako ELISA kit (minimum detection level 3 pmol/L, intra- and inter-assay coefficients of variation 5.1–7.5 % and 4.2–9.3% respectively) at the Department of Clinical Chemistry, Malmö University Hospital. Glucagon was assayed with RIA GL-32K (minimum detection level 18.5 pg/mL, intra- and inter-assay coefficients of variation 3.6–6.2 % and 8.7–14.7% respectively).

Statistical analysis

Continuous variables that were skewed were logarithmically transformed prior to analysis (at baseline: fasting blood glucose (FBG) and cystatin C; at re-examination: GIP pre and post challenge, GLP-1 pre and post challenge, insulin pre, during and post challenge, glucagon pre and post challenge, fasting plasma glucose (FPG) pre and post challenge, homeostasis model assessment of insulin resistance (HOMA-IR). Insulin resistance was estimated using HOMA-IR. (17) All analyses were performed in three steps according to *model 1* (adjusted for age, sex and follow-up time) and according to *model 2* (adjusted for age, sex, follow-up time, BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood glucose FBG) , beta-receptor blockers/ACE-inhibitors and kidney function (cystatin C), and further adjusted for full AHT (beta-receptor blockers, ACE-inhibitors, calcium antagonists, diuretics). Participants were divided in quartiles of HOMA-IR and were considered to have insulin resistance if their HOMA-IR measurement was in the top quartile (n=559). Logistic regression models were used to calculate odds ratios (OR) for the association of 1 SD of ANP increment and the upper quartile of HOMA-IR. Linear regression models were used to calculate effect sizes (β coefficients) of each 1 SD increment of baseline ANP on each 1 SD decrement of HOMA-IR at follow up. Further, linear regression models were used to calculate effect sizes of each 1 SD increment of ANP on 1 SD change of GIP, GLP-1, glucagon, FPG and insulin pre and post OGTT challenge.

All analyses were performed in SPSS Windows 22.0 (SPSS Inc, Illinois, USA). A two-tailed p value <0.05 was considered statistically significant. The median (interquartile range, IQR) follow-up time was 16.5 years.

Results

Baseline characteristics of the study population within quartiles of MR-proANP are listed in Table 1. Characteristics of the study population at follow-up within quartiles of MR-proANP are listed in Table 2.

Midlife exposure of ANP association with measurements of insulin resistance at follow-up

Results from linear regression analyses in *model 1* (adjusted for age, sex, and follow-up time) showed that each 1 SD increase of MR-proANP levels at baseline was highly significantly associated with lower HOMA-IR at follow-up (per 1 SD decrement of HOMA-IR: β=-0.080; p-value $3x10^{-4}$). In *model 2*, additionally adjusted for BMI, SBP, DBP, FBG, beta-receptor blockers/ACE-inhibitors and kidney function measured as Cystatin C, each 1 SD increment of MR-proANP was associated with lower risk of insulin resistance (per 1 SD decrement of HOMA-IR, β= -0.066, p-value 0.001), and remained so after additional adjustment for full AHT (β= -0.067; p-value 0.001). A logistic regression model demonstrated that each 1 SD of increase of MR-proANP at baseline was associated with decreased risk of belonging to upper quartile of HOMA-IR at follow-up (OR 0.88; CI 95% 0.78-0.98; p-value 0.018) when adjusted according to *model 1*. The association between MR-proANP and HOMA-IR remained significant after further adjustment according to *model 2* (OR 0.88; CI 95% 0.78-0.99; p-value 0.043). The results remained significant after full AHT adjustment (OR 0.88; CI 95% 0.78 – 0.99; p-value 0.035).

Furthermore, to explore whether any relationship between MR-proANP and insulin resistance was linear, MR-proANP levels were divided into quartiles and were related to top quartile HOMA-IR in a logistic regression model. Quartile analyses according to *model 1* revealed that subjects in the third (OR 0.60; CI 95% 0.45 – 0.79; p-value=$3x10^{-4}$) and fourth quartile (OR 0.67; CI 95% 0.51 – 0.90; p-value=0.008) of MR-proANP were at similarly low risk of insulin resistance, proposing protective

effects just above the median-value of MR-proANP. The association remained after further adjustment according to *model 2* (third quartile: OR 0.64; CI 95% 0.47 – 0.86; p-value 0.004; and fourth quartile: OR 0.69; CI 95% 0.51 – 0.96; p-value=0.025) as shown in Table 3. Additional adjustment for full AHT remained significant (p-value for trend 0.007) and showed similar results (data not shown). The HOMA-IR distribution within baseline quartiles of MR-proANP is illustrated in Figure 1 (p-value=0.002).

Interaction analysis in both linear (p-value 0.668) and logistic models (p-value 0.883) showed that the effects of ANP on insulin resistance are independent of baseline glucose levels. In order to assess the discrimination of MR-proANP values for risk of insulin resistance, a Receiver Operating Characteristic (ROC)-analysis was performed showing Area Under The Curve (AUC) 0.544 (CI 95% 0.516 – 0.572; p-value 0.002).

Midlife exposure of ANP and associations with OGTT responses of plasma levels of GIP, GLP-1, glucagon, glucose and insulin

Further analyses regarding the possible mechanisms behind this association revealed that higher ANP exposure (each 1 SD increment of MR-proANP) at baseline was associated with greater GIP release (per 1 SD increment: β=0.066; p-value=0.005) 120 minutes after 75g glucose intake, but not with GLP-1 release (per 1 SD increment: β=0.032; p-value=0.163) 120 minutes after 75g glucose intake at 16.5 years of follow up in *model 1*. The association remained significant after further adjustment according to *model 2*: β=0.055; p-value=0.020 per 1 SD increment of MR-proANP and 1 SD increment of GIP release (Table 4); additional adjustment for full AHT did not notably alter the association (β=0.052; p-value 0.026).

Additional analyses revealed that higher levels of baseline MR-proANP (each 1 SD increment of MR-proANP) were associated with lower fasting insulin secretion (per 1 SD decrement: β=-0.090; p-value=$2x10^{-4}$) and insulin secretion 30 minutes (per 1 SD decrement: β= -0.072; p-value=0.003) and 120 minutes (per 1 SD increment: β= -0.086; p-value=$4x10^{-4}$) after 75 g glucose intake in *model 1*. Those associations remained significant when adjusted according to *model 2* as shown in Table 5.

Further adjustment for full AHT did not markedly alter the associations (fasting insulin: β= -0.082; p-value=$2x10^{-4}$, insulin at 30 minutes: β= -0.082; p-value=0.001; insulin at 120 minutes: β= -0.081; p-value=$4x10^{-4}$). Each 1 SD increment in baseline levels of MR-proANP was also associated with higher fasting glucagon levels (per 1 SD increment: β=0.055; p-value=0.016) and higher glucagon secretion (per 1 SD increment: β=0.071; p-value=0.002) 120 minutes after 75g glucose intake in *model 1*. The associations remained significant when adjusted according to *model 2* (Table 5). Further adjustment for full AHT did not markedly alter the associations (fasting glucagon, per 1 SD increment: β=0.046; p-value 0.046; glucagon at 120 minutes, per 1 SD increment: β=0.058; p-value 0.014). No significant associations were found for fasting plasma glucose (per 1 SD increment: β= -0.023; p-value=0.328), nor for plasma glucose levels (per 1 SD increment: β= -0.042; p-value=0.080) 120 minutes after 75 g glucose intake.

Discussion

The key finding of our study is that a midlife exposure to ANP within the high normal range is associated with lower risk of insulin resistance at the reexamination after 16.5 years follow-up. Furthermore, we could also show that higher ANP levels at baseline examination were associated with a greater post OGTT-challenge GIP release at re-examination. This observation could explain the proposed insulin sensitizing effects of ANP seen in our study through previously demonstrated blood glucose stabilizing effects of GIP. (18) Since subjects with insulin resistance are at greater risk of developing T2D, (19) these data might serve as a mechanistic explanation for our previously published prospective data, showing that baseline ANP within the high normal range is protective against the development of diabetes after 16-years follow-up, (6) and also that a single nucleotide polymorphism rs5068 on the natriuretic peptide precursor A (NPPA) which genetically determined higher levels of ANP, is associated with lower risk of incident diabetes within 14 years. (8) Further, Xanthakis *et al*. demonstrated recently that natriuretic peptides are prospectively associated with ideal cardiovascular health in a middle-aged population. (20)

The ROC-analysis performed in order to assess the discrimination of MR-proANP values for risk of insulin resistance was modest and the association, although significant, was weak; however, the analysis of MR-proANP as a screening tool for subjects at risk of developing insulin resistance is beyond the scope of this study. Instead, our aim was to give a mechanistic explanation for our previously shown association between low ANP levels and the increased risk of incident diabetes. (6)

Earlier experimental data proposed that low ANP levels predispose diabetes to development and insulin resistance through an activation of the renin-angiotensin system. (21) Also, ANP has been demonstrated to exert beneficial effect on pancreatic beta cells. (4,22) As insulin plays a crucial role in diabetes metabolism, previous studies explored the association of NP's and β-cells. Natrium peptide receptor (NPR)-A knock out mice present reduced β-cell mass, higher fasting glucose levels and lower insulin levels in freshly isolated islets, and in vitro ANP have been shown to promote insulin secretion through blockade of ATP sensitive K1 channels. (23)

Our further findings on association of higher midlife exposure to ANP and lower insulin levels at follow-up, both fasting and during OGTT, are in line with the findings of lower prevalence of insulin resistance, as insulin resistance is characterized by loss of insulin sensitivity and a compensatory hyperinsulinemia. (24) Individuals with insulin resistance are at increased risk of developing T2D. (19) An association between lower levels of ANP and insulin resistance has previously been demonstrated in cross sectional studies, suggesting that low levels of NP's are a consequence of insulin resistance. (5) However, other studies, human, genetic and experimental, suggest that NP deficiency help promote insulin resistance. (25) Furthermore, ANP inhibits the secretion of factors involved in inflammation and insulin resistance such as IL-6 and TNF-α. (26) Our prospective study supports the notion that NP deficiency is most likely the cause of insulin resistance, rather than the reverse scenario, although we acknowledge that the effect sizes are modest.

ANP levels increase with age and are higher in women. (27) Our population consists of 61% women, and elderly subjects free from diabetes. By adjusting for age, sex and BMI we tried to eliminate possible confounding factors, since GIP secretion is positively correlated with BMI. Since GIP

secretion is near normal in diabetes, but its effect on insulin secretion is impaired, and GLP-1 secretion is impaired but the effect on insulin secretion preserved, it seems reasonable that both incretins are involved in pathogenesis of T2D. (28) Both incretins are measureable in fasting healthy subjects, indicating a basal secretion. However, it is the post challenge GIP and GLP-1 response to glucose intake that is crucial to glucose control. Studies comparing GIP response in healthy subjects versus subjects with diabetes demonstrated an enhanced GIP response in patients with diabetes (29), probably as a compensatory mechanism due to loss of GIP effect in diabetes; other studies demonstrated a GIP and GLP-1 response similar to those of healthy controls in subjects with relatively short T2D duration. (30) Since GIP acts as a blood glucose stabilizer, (18) higher GIP levels should result in better glucose control in healthy subjects. As HOMA-IR is reliable only in subjects free from diabetes, and GIP and GLP-1 secretion is deranged in diabetes, subjects with prevalent diabetes at baseline and incident diabetes at follow up were excluded from the study.

ANP is an instable hormone that undergoes rapid degradation in plasma. For this reason, several immunoassays that target more stable N-terminal fragments of the prohormones have been developed. (2,31) Those peptide fragments have decreased (if any) biological activity, but are widely used as surrogate markers of the biologically active peptides. Hence, even though the approach used in our study (the analysis of the mid-regional portion of the N-terminal fragment of ANP because MR-proANP is more stable that the fully processed ANP) may more reliably reflect ANP secretion, one must bear in mind that the measurements of MR-proANP do not necessarily reflect actual levels of fully processed ANP. In addition, both NP's are increased in subjects with heart failure, but their fragments are even more notably increased. (32) As we included participants with incident heart failure within the population, we cannot exclude the possibility that NP elevation caused by incident ischemic heart disease and/or heart failure (1,33), could have affected our results. However, the baseline MR-proANP values of our population are within the normal ANP-range, which argues against any symptomatic/asymptomatic alteration of cardiac function on average at baseline. Furthermore, in heart failure, there is evidence of the "natriuretic paradox", where a negative correlation between natriuretic peptide levels and BMI is observed, possibly explained by the frequent

treatment of obese subjects for hypertension and coronary artery disease, which reduces plasma levels of NPs. (34)

We tried to adjust for the known confounding factors; however, there is always a possibility of unmeasured rest confounding, e.g due to estrogen replacement therapy in peri-menopausal women. (35)

Further, we cannot exclude that proANP in plasma samples stored at -80°C from 1991-1996 until 2007, which is a considerable amount of time, might show some instability. This could represet a limitation to our study, considering that there is no data up to date on proANP stability and degradation after that amount of storage time.

There is increasing amount of evidence that the heart is an endocrine organ, and as such, involved in several metabolic processes. A recent review (36) demonstrates that NPs play an important role in control of energy usage and both ANP and BNP have been demonstrated to have lipolytic properties. It further describes that ANP has possible favorable effects on chronic inflammation, as it inhibits the release of adipokines and cytokines in human adipose tissue and increases lipid oxidation and the oxidative capacity of human skeletal muscle. In addition, NPs seem to enhance white adipose tissue browning.

NP's role in metabolic processes has been consolidated by several cross-sectional studies. As early as 1993, Clark *et al*. reported a significant raise in ANP-levels during hyperglycemia. (37) In 1994, Böhlen *et al*. reproduced those results. (38) More recent studies demonstrate that NP levels are reduced in subjects with obesity, insulin resistance and type 2 diabetes. (5,39) Reduced NP response is also associated with the activation of the renin-angiotensin system in experimental studies, an association that could partly explain the inversed association of NPs and the metabolic syndrome/insulin resistance. (40)

Kim et al. defined a gut-heart GLP-1 receptor-dependent and ANP-dependent axis that regulates blood pressure through promotion of ANP secretion in mice, (13) but another study on 12 healthy human subjects did not show any increase in either ANP or BNP concentrations during GLP-1 infusion. (14)

Neither our study did show any significant effects of baseline ANP-levels at post challenge GLP-1 release at follow-up.

Metabolic processes are complex and there are several mechanisms involved in diabetes development. However, there is increasing evidence that the clinical problem of frequent clustering of diabetes, hypertension and cardiovascular disease might have one common link: long term NP deficiency.

Conclusions

Midlife exposure to ANP within the high normal range is associated with lower risk of insulin resistance after 16.5 years of follow-up. Further, midlife exposure to ANP within the high normal range is associated with greater post challenge GIP secretion at follow-up, possibly explaining the lower prevalence of insulin resistance.

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Table 1. Baseline characteristics of the study population within quartiles of MR-proANP

	Total population (n=2243)	**MR-proANP Q1 (n=562)**	**MR-proANP Q2 (n=559)**	**MR-proANP Q3 (n=563)**	**MR-proANP Q4 (n=559)**
Age (years)	56.3 (±5.7)	54.0 (±5.2)	55.4 (±5.4)	57.2 (±5.6)	58.8 (±1.5)
Sex (female; n (%))	1364 (60.8)	266 (47.3)	320 (57.2)	388 (68.9)	390 (69.8)
BMI (kg/m^2)	25.1 (±3.4)	25.3 (±3.2)	25.2 (±3.4)	25.0 (±3.4)	24.9 (±3.4)
MR-proANP (pmol/L)	66.1 (51.2-85.1)	43.0 (37.9-47.4)	58.5 (55.2-62.4)	74.8 (69.8-79.3)	103.0 (92.5-121.0)
Follow-up time (years)	16.6 (±1.5)	16.7 (±1.5)	16.6 (±1.5)	16.6 (±1.5)	16.5 (±1.5)
SBP (mmHg)	138.4 (±17.6)	136.6 (±15.9)	136.7 (±16.9)	137.6 (±17.7)	142.6 (±19.2)
DBP (mmHg)	85.8 (±8.9)	86.4(±8.2)	85.6 (±8.7)	84.7 (±8.9)	86.4 (±9.9)
FBG (mmol/L)	4.8 (4.5-5.1)	4.9 (4.6-5.2)	4.8 (4.5-5.1)	4.8 (4.5-5.0)	4.7 (4.5-5.1)
AHT (n (%))	177 (7.9)	22 (3.9)	32 (5.7)	37 (6.6)	86 (15.4)
Cystatin C (mg/L)	0.75 (0.67-0.82)	0.73 (0.66-0.80)	0.74 (0.67-0.81)	0.75 (0.69-0.83)	0.77 (0.69-0.87)

Values are means (±SD) or median (25-75 interquartile range (IQR)). BMI=body mass index; MR-proANP=mid-regional pro atrial natriuretic peptide; SBP=systolic blood pressure; DBP=diastolic blood pressure; FBG=fasting blood glucose; AHT=antihypertensive treatment; Q1=quartile with the lowest values of MR-proANP; Q4=quartile with the highest values of MR-proANP

Table 2. Characteristics of the study population at follow-up within baseline quartiles of MR-proANP

	Total population (n=2243)	MR-proANP Q1 (n=562)	MR-proANP Q2 (n=559)	MR-proANP Q3 (n=563)	MR-proANP Q4 (n=559)
Age (years)	72.4 (±5.6)	70.2 (±5.2)	71.5 (±5.4)	73.2 (±5.5)	74.8 (±5.2)
Sex (female)	1364 (60.8)	266 (47.3)	320 (57.2)	388 (68.9)	390 (69.8)
BMI (kg/m^2)	26.5 (±4.1)	26.9 (±3.7)	26.6 (±4.1)	26.4 (±4.2)	26.2 (±4.3)
Follow-up time (years)	16.6 (±1.5)	16.7 (±1.5)	16.6 (±1.5)	16.6 (±1.5)	16.5 (±1.5)
HOMA-IR	1.96 (1.30-2.84)	2.14 (1.37-3.10)	2.03 (1.36-2.88)	1.79 (1.27-2.59)	1.90 (1.3-2.8)
GIP (pmol/L)*	39.9 (29.7-53.5)	41.1 (28.7-55.2)	38.8 (28.7-51.1)	39.8 (30.1-53.6)	40.0 (31.5-54.4)
GIP (pmol/L)‡	224.3 (165.7-295.5)	207.2 (158.5-273.1)	219.2 (160.4-289.5)	219.5 (165.6-299.1)	246.2 (183.1-321.9)
GLP-1 (pmol/L)*	8.0 (6.0-10.0)	8.0 (6.0-10.0)	8.0 (6.0-10.0)	8.0 (6.0-9.0)	8.0 (6.00-10.00)
GLP-1 (pmol/L)‡	16.0 (12.0-21.0)	15.0 (11.0-19.0)	15.0 (12.0-20.0)	16.0 (12.0-21.0)	17.0 (13.0-22.0)
Insulin (pmol/L)*	7.6 (5.3-10.7)	8.2 (5.5-11.5)	8.0 (5.6-10.7)	7.0 (5.1-9.8)	7.2 (5.2-10.5)
Insulin (pmol/L)†	42.2 (29.5-60.9)	45.6 (30.4-65.3)	43.6 (31.1-62.0)	40.0 (28.5-56.5)	41.2 (28.5-59.7)
Insulin (pmol/L)‡	40.0 (25.7-63.2)	41.0 (26.1-67.3)	42.1 (26.1-63.2)	36.9 (23.9-59.2)	39.0 (26.3-65.6)
Glucose (mmol/L)*	5.8 (5.4-6.2)	5.8 (5.4-6.3)	5.8 (5.4-6.2)	5.8 (5.4-6.3)	5.8 (5.4-6.3)
Glucose (mmol/L)‡	6.7 (5.4-8.2)	6.8 (5.4-8.3)	6.7 (5.3-8.0)	6.7 (5.4-8.2)	6.8 (5.6-8.2)
Glucagon (pg/mL)*	75.8 (64.0-90.0)	75.4 (63.0-90.4)	77.0 (65.0-90.0)	74.0 (63.0-89.0)	76.0 (64.0-90.2)
Glucagon (pg/mL)‡	70.0 (59.0-83.0)	68.4 (58.0-80.0)	69.8 (58.4-84.0)	69.0 (59.0-81.0)	73.0 (61.0-87.2)

Values are means (±SD) or median (25-75 interquartile range (IQR)). BMI=body mass index; MR-proANP=midregional atrial pro natriuretic peptide. *=Fasting levels; †=Levels at 30 minutes; ‡=Levels at 120 minutes; HOMA-IR=homeostatic model assessment of insulin resistance; GIP=glucose-dependent insulinotropic peptide; GLP-1= glucagon-like peptide-1; Q1=quartile with the lowest values of MR-proANP; Q4=quartile with the highest values of MR-proANP

Table 3. Quartile distribution of MR-proANP in relation to top quartile of HOMA-IR

MODEL 1	**OR (CI 95%)**	**p-value**
MR-proANP as a continuous variable	0.72 (0.55 – 0.95)	0.018
HOMA-IR as a categorical variable	**Quartiles of MR-proANP**	
Group 1 (lowest values)	Referent	
Group 2	0.78 (0.60 – 1.01)	0.066
Group 3	0.60 (0.45 – 0.79)	<0.001
Group 4 (highest values)	0.67 (0.51 – 0.90)	0.008
p for trend		<0.001

MODEL 2	**OR (CI 95%)**	**p-value**
MR-proANP as a continuous variable	0.74 (0.56 – 0.99)	0.043
HOMA-IR as a categorical variable	**Quartiles of MR-proANP**	
Group 1 (lowest values)	Referent	
Group 2	0.81 (0.61 – 1.08)	0.115
Group 3	0.64 (0.47 – 0.86)	0.004
Group 4 (highest values)	0.69 (0.51 – 0.96)	0.025
p for trend		0.009

Values are odds ratios (95% confidence intervals) for insulin resistance (top quartile of HOMA-IR). *Model 1* is adjusted for age, sex and follow-up time. *Model 2* is adjusted for age, sex, follow up time, body mass index, systolic blood pressure, diastolic blood pressure, use of antihypertensive medication, fasting blood glucose, cystatin C. Values of MR-proANP within quartiles, q (mean, (SD)): q1: 41.79 (±6.89), q2: 58.75 (±4.28), q3: 74.99 (±5.41) and q4: 112.68 (±34.49).

Table 4. Association of 1 SD increment of baseline MR-proANP with 1 SD change in incretin levels at follow-up pre (0 minutes) and post (120 minutes) OGTT challenge

	GIP 0		**GIP 120**		**GLP-1 0**		**GLP-1 120**	
	β	p	β	p	β	p	β	p
Age	0.08	0.045	0.025	<0.001	-0.001	0.813	0.028	<0.001
Sex	0.027	0.544	0.421	<0.001	-0.040	0.389	0.405	<0.001
BMI	0.021	0.001	-0.024	<0.001	0.006	0.378	-0.025	<0.001
Follow-up time	-0.053	<0.001	-0.063	<0.001	0.045	0.002	0.079	<0.001
SBP	-0.002	0.346	0.001	0.557	-0.002	0.268	0.000	0.819
DBP	0.004	0.265	0.005	0.120	0.003	0.465	0.003	0.410
FBG	0.013	0.959	0.014	0.956	-0.176	0.500	-0.672	0.007
AHT	-0.012	0.885	-0.048	0.535	0.082	0.317	0.121	0.122
Cystatin C	0.767	<0.001	0.406	0.002	0.036	0.797	-0.029	0.829
MR-proANP	0.005	0.823	0.055	0.020	0.004	0.857	0.016	0.493

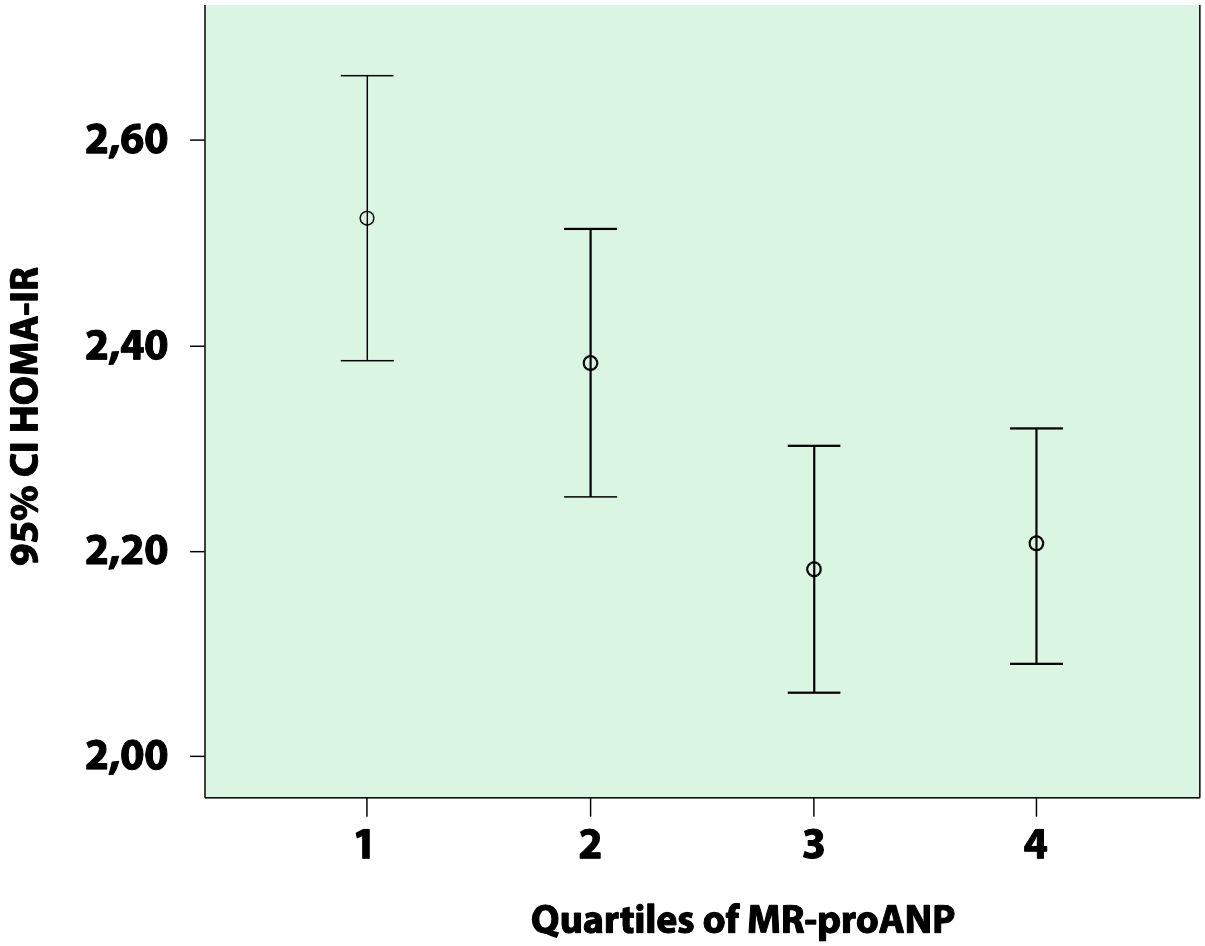
β are unstandardized coefficients. BMI=body mass index; MR-proANP=mid-regional pro atrial natriuretic peptide; SBP=systolic blood pressure; DBP=diastolic blood pressure; FBG=fasting blood glucose; AHT=antihypertensive treatment; GIP=glucose-dependent insulinotropic peptide; GLP-1=glucagon-like peptide-1.

Table 5. Levels of 1 SD change in insulin (pro, during and post challenge), glucose (pro and post challenge) and glucagon (pro and post challenge) levels related to 1 SD increment of baseline MR-proANP levels

	Insulin*		Insulin‡		Insulin†		Glucose*		Glucose†		Glucagon*		Glucagon†	
	β	p	β	p	β	p	β	p	β	p	β	p	β	p
Age	-0.005	0.168	-0.002	0.583	0.012	0.003	-0.001	0.810	0.022	<0.001	-0.005	0.231	0.005	0.193
Sex	0.063	0.129	0.149	0.001	0.289	<0.001	-0.008	0.847	0.150	0.001	-0.477	<0.001	-0.146	0.001
BMI	0.095	<0.001	0.060	<0.001	0.053	<0.001	0.023	<0.001	0.036	<0.001	0.037	<0.001	0.015	0.018
Follow-up time	0.012	0.344	0.027	0.056	0.026	0.061	-0.073	<0.001	-0.001	0.918	-0.123	<0.001	-0.148	<0.001
SBP	0.004	0.011	0.001	0.642	0.006	0.001	0.006	0.001	0.007	<0.001	0.004	0.021	0.002	0.234
DBP	0.004	0.248	0.004	0.261	0.007	0.024	0.002	0.563	0.000	0.941	0.000	0.926	0.002	0.430
FBG	1.286	<0.001	-0.039	0.122	1.500	<0.001	4.434	<0.001	2.145	<0.001	-0.107	0.657	-0.189	0.443
AHT	0.130	0.080	0.158	0.048	0.164	0.033	-0.023	0.750	0.113	0.143	0.046	0.543	0.016	0.842
Cystatin C	0.624	<0.001	0.492	<0.001	0.268	0.043	-0.090	0.471	-0.123	0.355	0.444	0.001	0.502	<0.001
MR-proANP	-0.082	<0.001	-0.080	0.001	-0.081	0.004	0.012	0.579	-0.028	0.230	0.048	0.037	0.061	0.014

β are unstandardized coefficients. BMI=body mass index; MR-proANP=mid-regional pro atrial natriuretic peptide; MR-proANP=mid-regional pro atrial natriuretic peptide; SBP=systolic blood pressure; DBP=diastolic blood pressure; FBG=fasting blood glucose; AHT=antihypertensive treatment; *=Fasting levels; ‡=Levels at 30 minutes; †=Levels at 120 minutes

Figure 1. Baseline quartiles of MR-proANP in relation to HOMA-IR at follow up



HOMA-IR at follow-up within quartiles of MR-proANP at baseline. HOMA-IR=homeostatic model assessment of insulin resistance; MR-proANP=mid-regional pro atrial natriuretic peptide