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RESEARCH ARTICLE

Sex-specific associations of obesity and N-terminal pro-B-type natriuretic peptide levels in the general population

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Background Obese subjects have lower natriuretic peptide levels, but males and females have different anthropometric characteristics and fat distribution. Whether obesity-associated lowering of natriuretic peptides differs among males and females is unknown. Therefore, we investigated sex-specific associations of obesity and N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels among adults in the general population.

Methods and results Using 8260 participants (50.1% females) from the Prevention of REnal and Vascular ENd-stage Disease (PREVEND) cohort, we evaluated the relationship of NT-proBNP levels with obesity-associated parameters, i.e. waist circumference (WC), body mass index (BMI) and body weight in the overall population, and in males and females separately. NT-proBNP levels were higher in females (median, interquartile range: 50.5, 28.2–87.0 ng/L) than in males (24.3, 10.1–54.6 ng/L; $P < 0.001$). In the overall population, NT-proBNP levels were significantly lower in heavier individuals and displayed a 'U-shaped' relationship with increasing WC, but were not associated with BMI. After sex stratification, there was no significant association between NT-proBNP concentrations and anthropometric measures in females. However, in males increasing WC and BMI were associated with higher NT-proBNP levels ($P < 0.05$) while increasing body weight was associated with slightly lower NT-proBNP levels ($P < 0.05$). Age strongly confounded the association of NT-proBNP levels with obesity, and age-associated increases in NT-proBNP were significantly higher in males than in females ($P < 0.001$). In multivariable adjusted analyses, the inverse association of obesity and NT-proBNP levels was also significantly modified by sex: NT-proBNP levels were lower with increasing WC, BMI and body weight among females compared with males ($P_{interaction} < 0.05$). After also accounting for BMI, abdominal obesity was associated with lower NT-proBNP levels in females, but not in males ($P_{interaction} < 0.001$).

Conclusions Natriuretic peptide deficiency in obesity mostly pertains to females with abdominal obesity, whereas the relationship between obesity and natriuretic peptides appears to be more complex in males.

Keywords NT-proBNP • Females • Males • Obesity • Sex • Age

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Introduction

N-terminal pro-B-type natriuretic peptide (NT-proBNP) is a widely used biomarker in diagnosing heart failure (HF), particularly in patients presenting with dyspnoea and other atypical complaints.[1−3] NT-proBNP is also used in risk stratification of several cardiovascular (CV) disorders.[4,5] It is considered to be more stable than BNP because of its relatively long half-life[6]; however, various factors influence NT-proBNP levels. Consistently elevated NT-proBNP levels are observed in older individuals[7,8] and also in patients with CV disorders and renal dysfunction; indeed, cardiac wall stress and (cardiac) hypoxic stimuli are considered to be key drivers of NT-proBNP release, and these peptides are primarily excreted by the kidneys.[1,9,10]

Females exhibit higher levels of NT-proBNP compared to males; up to a 100% difference in NT-proBNP levels has been observed in healthy individuals.[11] The reasons for these sex-specific differences are not fully understood, it has been proposed that sex hormones play a role.[12,13] Another important factor affecting NT-proBNP is obesity; previous studies report that individuals with higher body mass index (BMI) have lower natriuretic peptide levels compared to normal weight subjects.[14−16] However, sex-specific data regarding obesity-associated reduction of NT-proBNP remain scarce.

Since males and females have profoundly different anthropometric characteristics and fat distribution,[17,18] we hypothesized that low NT-proBNP levels observed in obese individuals could be attributed, at least in part, to differences in sex. We, therefore, studied the impact of sex on the relationship between NT-proBNP and obesity-associated parameters, i.e. waist circumference (WC), BMI, and body weight. All analyses were first performed in the combined population and then in males and females separately.

Methods

Study population

The Prevention of REnal and Vascular ENd-stage Disease (PREVEND) study (1997–1998) is a prospective Dutch cohort taken from the general population of Groningen, The Netherlands. All inhabitants of the city of Groningen ($n = 85\,421$) were invited to answer a postal questionnaire and to provide an early morning urine sample. The response rate was 47.8% ($n = 40\,856$), and urinary albumin and creatinine levels were measured in these respondents. Pregnant women (self-reported) and those with prevalent type 1 diabetes mellitus (i.e. those using insulin) were excluded. All remaining subjects with an urinary albumin excretion (UAE) of ≥10 mg/L ($n = 6000$) and a randomly selected control group with UAE <10 mg/L ($n = 2592$) underwent further investigation and constitute the baseline PREVEND cohort. The primary aim of this study was to prospectively evaluate the association of increased UAE with CV and renal disease in apparently healthy individuals.

PREVEND participants underwent baseline examination in an outpatient clinic. Urine samples were collected (two consecutive 24 h urine samples from each individual) and fasting venous blood samples were drawn into EDTA tubes, aliquoted and stored at −80 °C until analysis. NT-proBNP levels were measured in blood samples of 8383 subjects (obtained during the baseline visit) using an Elecsys™ 2010 analyser that uses an electrochemiluminescence sandwich immunoassay technique (Elecsys proBNP, Roche Diagnostics, Mannheim, Germany). This technique is based on two polyclonal antibodies, i.e. a biotinylated antibody and a ruthenium derivative-labelled antibody that bind to residues in NT-proBNP molecule. The intra-assay imprecision is around 1.2–1.5% whereas the inter-assay imprecision is around 4.4–5.0%; precision profile showed that the intra-assay imprecision is <10% across the entire analytical range (i.e. 5–35 000 ng/L) and is <3% at all concentrations >30 ng/L.[19] Plasma concentrations of NT-proBNP are given as ng/L; to convert to pmol/L divide by 8.46 (i.e. 100 ng/L = 11.8 pmol/L). Further details of methodology and assays relevant to this study can be found in the online supplementary *Methods S1*.

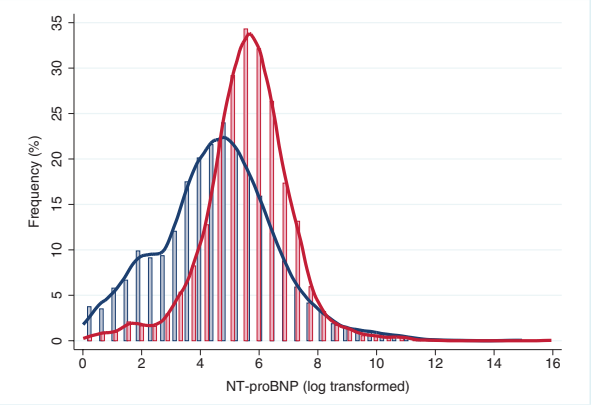

Figure 1 Distribution of N-terminal pro-B-type natriuretic peptide (NT-proBNP) in males and females. Histograms showing distribution of $\log_2$-transformed NT-proBNP levels in males (blue) and females (red). Values below the detection limit (i.e. 5 ng/L) were assigned random values between 1 ng/L and 5 ng/L.

From the baseline cohort ($n = 8592$), we first excluded patients with prevalent HF ($n = 23$). Another 309 participants were excluded due to incomplete data (absent NT-proBNP measurements or incomplete data recorded on obesity-related parameters), leaving 8260 participants for final analysis (online supplementary *Figure S1*). An in-depth description of the PREVEND study can be found elsewhere.[5,20,21] The PREVEND study was conducted according to the principles drafted in the Helsinki declaration. The local medical ethics committee approval was obtained and informed consent was provided by all participants.

Definitions

All measurements were performed during the baseline visit or with plasma samples obtained during the baseline visit. Anthropometric parameters (i.e. WC, BMI and body weight) were measured in a standing position. WC was measured midway between the lowest rib and the iliac crest, at the end of expiration. Hip circumference was measured at the widest portion at the level of the greater trochanters. BMI was calculated as the ratio between weight and height squared (kg/m^2). Hypercholesterolaemia was defined as total serum cholesterol ≥6.5 mmol/L (251 mg/dL) or a serum cholesterol of ≥5.0 mmol/L (193 mg/dL) if a history of myocardial infarction (MI) was present or when lipid-lowering medication was used. Blood pressure was measured 10 times during 10 min using an automatic Dinamap XL Model 9300 series; blood pressure was calculated as the mean of the last two measurements. Hypertension was defined as systolic blood pressure > 140 mmHg, diastolic blood pressure > 90 mmHg, or self-reported antihypertensive medication usage. History of MI was based on subject's medical history derived from

Table 1 Baseline characteristics according to tertiles of waist circumference in the combined population

	Tertile (males + females)			*P*-value
	1 ($n = 2797$)	2 ($n = 2789$)	3 ($n = 2674$)	
Waist circumference, cm	74.5 (5.3)	88.5 (3.5)	103.4 (7.6)	<0.001
NT-proBNP, ng/L	42.8 (23.3–75.4)	31.3 (13.9–66.3)	35.5 (14.0–78.3)	<0.001
Female sex, *n* (%)	2185 (78.1)	1173 (42.1)	782 (29.2)	<0.001
Age, years	43.5 (10.8)	49.5 (12.6)	55.1 (11.8)	<0.001
Smoking (last 1 year), *n* (%)	1203 (43.1)	1037 (37.3)	888 (33.3)	<0.001
Hypertension, *n* (%)	292 (10.4)	734 (26.3)	1227 (46.9)	<0.001
Diabetes mellitus, *n* (%)	26 (1.0)	73 (2.7)	213 (8.2)	<0.001
Hypercholesterolaemia, *n* (%)	450 (16.3)	803 (29.2)	926 (35.1)	<0.001
Myocardial infarction, *n* (%)	103 (3.7)	145 (5.3)	244 (9.3)	<0.001
Body mass index, kg/m^2	22.8 (2.4)	25.8 (2.5)	29.9 (4.0)	<0.001
Weight, kg	65.8 (8.0)	77.9 (8.0)	91.7 (12.4)	<0.001
Total cholesterol, mmol/L	5.2 (4.6–5.9)	5.7 (4.9–6.4)	5.9 (5.2–6.5)	<0.001
HDL, mmol/L	1.5 (1.3–1.8)	1.2 (1.0–1.5)	1.1 (0.9–1.3)	<0.001
Systolic BP, mmHg	118.2 (16.6)	130.4 (18.7)	139.1 (19.4)	<0.001
Glucose, mmol/L	4.4 (4.1–4.8)	4.7 (4.4–5.1)	5.0 (4.6–5.6)	<0.001
Insulin resistance (HOMA-IR)	1.2 (0.9–1.6)	1.7 (1.2–2.4)	2.7 (1.8–4.2)	<0.001
hs-CRP, mg/L	0.7 (0.3–1.9)	1.2 (0.6–2.7)	2.2 (1.1–4.5)	<0.001
eGFR (mL/min/1.73 m^2)	100.6 (15.1)	94.8 (17.1)	89.3 (17.7)	<0.001

BP, blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitive C-reactive protein; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

a structured questionnaire (i.e. hospitalization ≥3 days as a result of this condition); this was complemented by a review of the medical report. Insulin resistance was calculated using the homeostasis model assessment-estimated insulin resistance (HOMA-IR) method. Type 2 diabetes mellitus was defined as a fasting plasma glucose ≥7.0 mmol/L (126 mg/dL), random plasma glucose ≥11.1 mmol/L (200 mg/dL), self-reporting of a physician diagnosis, or record of glucose-lowering medication use obtained from central pharmacy registry. Smoking was defined as self-reported current smoking, or smoking cessation within the previous year. Glomerular filtration rate was estimated using the simplified Modification of Diet in Renal Disease formula.

Statistical analyses

Normally distributed data are presented as means ± standard deviations (SD) and non-normally distributed data are presented as medians ± interquartile ranges (IQR). Categorical variables are represented as percentages. Skewed variables were $\log_2$ transformed in order to facilitate interpretation.

All analyses were performed in the overall population, and also separately in males and females. ANOVA was used to test for differences between groups with normally distributed data, and Kruskal–Wallis test was used for non-normally distributed data. Differences between categorical variables were tested using Pearson's chi-square test. Associations between $\log_2$-transformed NT-proBNP and obesity-associated parameters were evaluated using univariable linear regression analysis and a multivariable regression model that also adjusted for factors known to influence NT-proBNP levels. Results are displayed as standardized β coefficient (Sβ), which represents the SD change in dependent variable for 1 SD change in the independent variable. R^2 represents the coefficient of determination, and can be interpreted as the proportion of variation in the dependent variable that is predictable from one or more independent variables.

Univariable associations of anthropometric parameters and age with median NT-proBNP levels were graphically modelled using kernel-weighted local polynomial smoothing. Multivariable-adjusted associations of WC with NT-proBNP levels were graphically assessed by means of regression analysis using a restricted cubic spline function to check for possible deviations from linearity. As the PREVEND cohort had an overrepresentation of subjects with increased UAE, we performed a sensitivity analysis by further adjusting for urinary albumin levels to test for robustness of output. All statistical analyses were performed using STATA version 14 (Stata Corp., College Station, TX, USA), and a *P*-value of ≤0.05 was considered to be significant.

Results

Plasma NT-proBNP levels

The study included 8260 participants that were free of HF at baseline, and 50.1% ($n = 4140$) were females. The mean age of the overall population was 49.3 ±12.7 years. The median NT-proBNP level in the overall population was 37.5 ng/L (IQR 16.7–73.5 ng/L); the range varied from ≤5 ng/L ($n = 732$) to ≥35 000 ng/L ($n = 1$). NT-proBNP levels were significantly higher in females than in males (median, IQR: 50.5, 28.2–87.0 vs. 24.3, 10.1–54.6 ng/L, $P < 0.001$) (*Figure 1*). The mean age in females was slightly (yet significantly) lower than in males (48.2 ±12.3 vs. 50.4 ±12.9 years; $P < 0.001$).

Baseline characteristics

We divided the entire population and the sex-specific populations in tertiles of WC to provide initial insights into the association of body fat composition with NT-proBNP and other clinical characteristics. WC is also a better surrogate marker of visceral

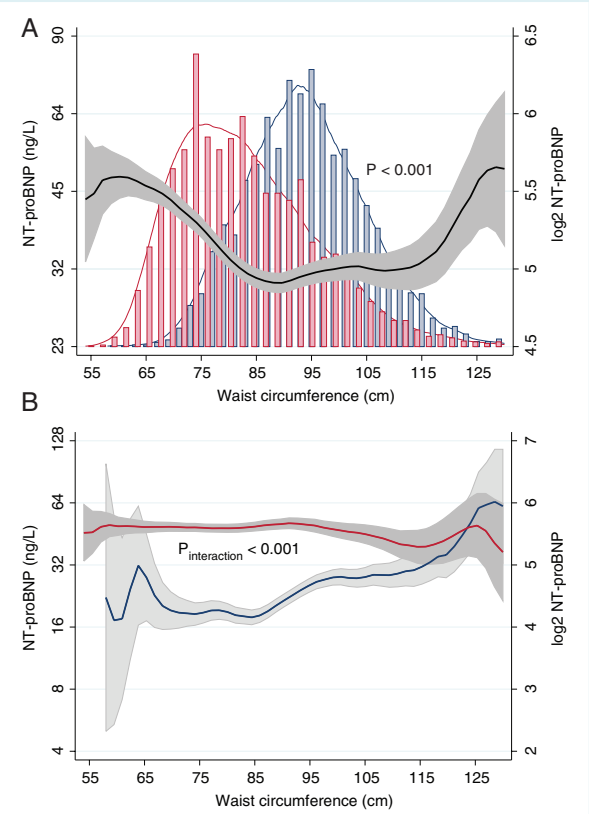

Figure 2 (*A*) Association of waist circumference and N-terminal pro-B-type natriuretic peptide (NT-proBNP) in the overall population using kernel-weighted local polynomial smoothing graphic modelling. Black lines represent median NT-proBNP (ng/L) levels in the overall population; grey bands represent prediction intervals of median NT-proBNP; histograms represent distribution of waist circumference in males (blue) and females (red); waist circumference ≤ 130 cm. (*B*) Associations of waist circumference and NT-proBNP levels in males and females. Blue lines represent median NT-proBNP (ng/L) levels in males; red lines represent median NT-proBNP (ng/L) levels in females; grey bands represent prediction intervals of median NT-proBNP; waist circumference ≤ 130 cm.

adipose tissue, which represents a metabolically active adipose tissue compartment.[22–26]

NT-proBNP displayed a 'U-shaped' relationship with increasing WC (*Table 1*, *Figure 2A*). Subjects with higher WC were usually older and more often males. They also had a more frequent history of hypertension, diabetes mellitus, hypercholesterolaemia and MI. As expected, increase in WC strongly correlated with higher BMI, body weight and total cholesterol, and inversely correlated with high-density lipoprotein levels. Individuals with larger WC also exhibited increased insulin resistance, signs of low-grade systemic inflammation, and reduced renal function (*Table 1*). Stratification by sex revealed similar associations with all parameters, except for NT-proBNP: males with higher WC had higher NT-proBNP levels, but no significant association was found in females ($P_{interaction} < 0.001$) (*Table 2*, *Figure 2B*).

We further explored these associations by tabulating baseline characteristics according to tertiles of BMI and body weight; similar trends were observed for all parameters, except for NT-proBNP (online supplementary *Tables S1* and *S2*). In the overall population, increasing BMI was not associated with NT-proBNP levels. Sex-specific analysis revealed that males with higher BMI had higher NT-proBNP levels, while increasing BMI was not associated with higher NT-proBNP in females ($P_{interaction} = 0.001$) (online supplementary *Figure S2*).

Higher body weight correlated strongly with lower NT-proBNP levels in the overall population ($P < 0.001$). Sex stratification significantly modified this negative relationship: heavier males had (slightly) lower NT-proBNP levels, and increasing body weight was not associated with NT-proBNP levels in females. Interaction analysis revealed there was no significant difference in the association of body weight with NT-proBNP levels in males and females ($P_{interaction} = 0.720$) (*Figure 3*).

Impact of sex and age on NT-proBNP levels

We characterized the relationship of sex and age with NT-proBNP levels using linear regression models. Female sex was associated with higher NT-proBNP levels ($S\beta = 0.286$, $P < 0.001$), and sex accounted for approximately 8.2% of the model variance in the overall population. Higher age was also associated with higher NT-proBNP levels in the overall population ($P < 0.001$), and age explained 13.0% of the model variance (*Table 3*). The relationship between age and median NT-proBNP levels is also graphically depicted in *Figure 4A*.

When we evaluated the relationship of age with NT-proBNP sex-specifically, there was a very strong and positive association between age and NT-proBNP levels in males ($S\beta = 0.520$, $P < 0.001$). Females also exhibited a significant positive correlation with age, albeit weaker ($S\beta = 0.245$, $P < 0.001$). Age-related increases in NT-proBNP were clearly higher in males than in females ($P_{interaction} < 0.001$) (*Figure 4B*). Age explained about 27.0% of the model variance in males while it accounted for only 6.0% of the model variance in females.

Associations of anthropometric parameters with NT-proBNP

We then sex-stratified our analyses and evaluated the association of anthropometric parameters with NT-proBNP levels using a linear regression model that adjusted for age (model 1) and a multivariable adjusted model that also accounted for other covariates known to affect NT-proBNP levels (model 2).[12]

After accounting for age in model 1, all obesity parameters displayed an inverse relationship with NT-proBNP levels in both males and females. Increasing WC, BMI and body weight were significantly associated with lower NT-proBNP levels in females compared with males ($P_{interaction} < 0.05$) (*Table 4*).

Other factors affecting NT-proBNP include previous MI, renal dysfunction, hypertension including antihypertensive medication usage, insulin resistance, and low-grade systemic inflammation.[12,27]

Table 2 Baseline characteristics according to tertiles of waist circumference in males and females

Characteristic	Tertile (males)			*P*-value	Tertile (females)			*P*-value
	1 ($n = 1437$)	2 ($n = 1354$)	3 ($n = 1329$)		1 ($n = 1406$)	2 ($n = 1362$)	3 ($n = 1372$)	
Waist circumference, cm	82.4 (5.2)	93.7 (2.5)	106.4 (6.8)	<0.001	70.6 (3.9)	81.7 (3.2)	98.0 (8.8)	<0.001
NT-proBNP, ng/L	19.1 (9.1–38.6)	26.3 (9.8–62.2)	29.7 (11.1–70.7)	<0.001	51.0 (30.1–86.2)	48.7 (28.6–81.6)	51.7 (26.9–95.6)	0.350
Age, years	44.1 (11.6)	51.7 (12.8)	55.8 (11.5)	<0.001	42.4 (10.1)	48.2 (11.7)	54.1 (12.1)	<0.001
Smoking (last 1 year), *n* (%)	624 (43.5)	503 (37.3)	439 (33.2)	<0.001	626 (44.7)	501 (36.9)	435 (31.8)	<0.001
Hypertension, *n* (%)	206 (14.3)	461 (34.0)	693 (52.2)	<0.001	120 (8.5)	257 (18.9)	516 (37.6)	<0.001
Diabetes mellitus, *n* (%)	24 (1.8)	44 (3.4)	111 (8.7)	<0.001	7 (0.5)	22 (1.7)	104 (7.7)	<0.001
Hypercholesterolaemia, *n* (%)	254 (17.9)	404 (30.2)	455 (35.8)	<0.001	205 (14.7)	356 (26.4)	505 (37.4)	<0.001
Myocardial infarction, *n* (%)	72 (5.1)	116 (8.7)	130 (10.0)	<0.001	42 (3.0)	54 (4.1)	78 (5.8)	<0.001
Body mass index, kg/m^2	23.2 (2.1)	26.1 (1.9)	29.9 (3.1)	<0.001	22.0 (2.0)	25.4 (2.4)	30.5 (4.6)	<0.001
Weight, kg	74.4 (8.0)	83.7 (7.4)	96.1 (11.6)	<0.001	61.9 (6.8)	70.7 (7.2)	83.8 (12.8)	<0.001
Total cholesterol, mmol/L	5.3 (4.7–6.1)	5.7 (5.0–6.4)	5.8 (5.3–6.5)	<0.001	5.1 (4.5–5.8)	5.6 (4.8–6.4)	5.9 (5.2–6.6)	<0.001
HDL, mmol/L	1.2 (1.0–1.5)	1.1 (0.9–1.3)	1.0 (0.9–1.2)	<0.001	1.6 (1.4–1.9)	1.5 (1.2–1.7)	1.3 (1.1–1.5)	<0.001
Systolic BP, mmHg	125.6 (15.2)	134.2 (17.8)	142.0 (18.7)	<0.001	115.0 (16.0)	123.7 (19.6)	134.6 (21.9)	<0.001
Glucose, mmol/L	4.6 (4.3–5.0)	4.8 (4.5–5.2)	5.1 (4.7–5.6)	<0.001	4.4 (4.0–4.7)	4.6 (4.2–4.9)	4.9 (4.5–5.5)	<0.001
Insulin resistance (HOMA-IR)	1.2 (0.9–1.8)	1.8 (1.3–2.6)	3.0 (1.9–4.4)	<0.001	1.1 (0.8–1.5)	1.5 (1.1–2.1)	2.5 (1.7–4.0)	<0.001
hs-CRP, mg/L	0.7 (0.3–1.6)	1.2 (0.6–2.7)	2.0 (1.1–3.9)	<0.001	0.7 (0.3–1.7)	1.2 (0.6–2.9)	2.6 (1.3–5.2)	<0.001
eGFR (mL/min/1.73 m^2)	100.8 (15.5)	93.0 (17.4)	88.8 (17.8)	<0.001	101.1 (14.3)	96.5 (16.3)	89.3 (17.9)	<0.001

BP, blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitive C-reactive protein; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

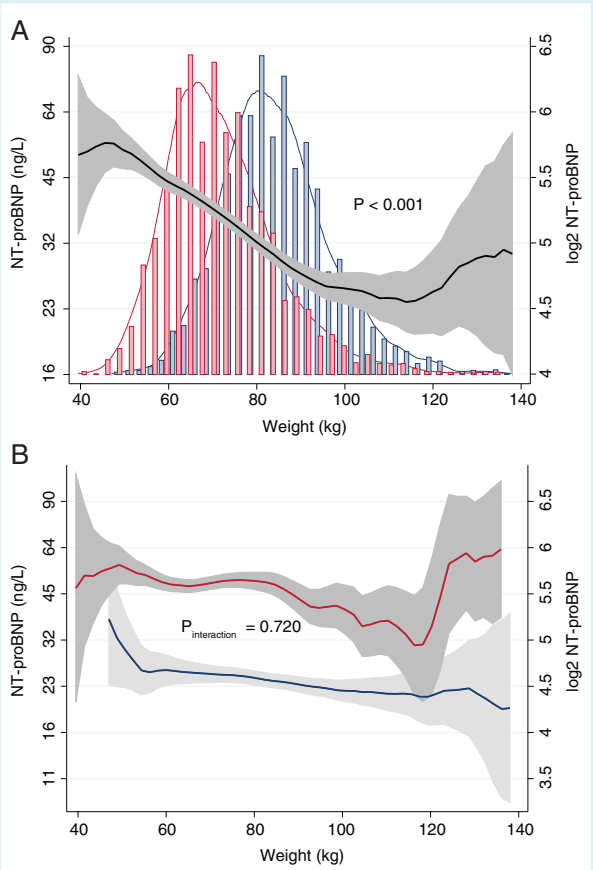

Figure 3 (*A*) Association of body weight and N-terminal pro-B-type natriuretic peptide (NT-proBNP) in the overall population using kernel-weighted local polynomial smoothing graphic modelling. Black lines represent median NT-proBNP (ng/L) levels in the overall population; grey bands represent prediction intervals of median NT-proBNP; histograms represent distribution of body weight in males (blue) and females (red); body weight ≤ 140 kg. (*B*) Associations of body weight and NT-proBNP levels in males and females. Blue lines represent median NT-proBNP (ng/L) levels in males; red lines represent median NT-proBNP (ng/L) levels in females; grey bands represent prediction intervals of median NT-proBNP; body weight ≤ 140 kg.

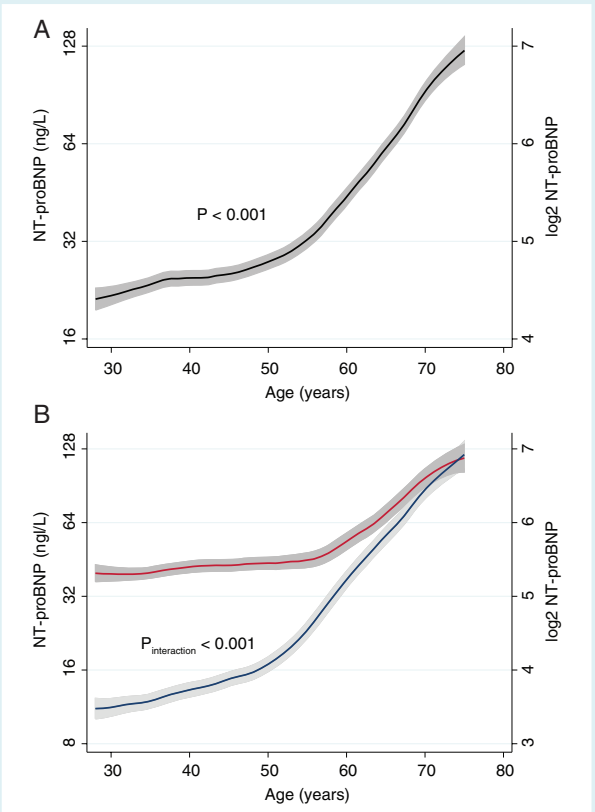

Figure 4 (*A*) Associations of age and N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels in the combined population using kernel-weighted local polynomial smoothing graphic modelling. Black lines represent median NT-proBNP (ng/L) levels in the overall population; grey bands represent prediction intervals of median NT-proBNP. (*B*) Associations of age and NT-proBNP levels in males and females. Blue lines represent median NT-proBNP (ng/L) levels in males; red lines represent median NT-proBNP (ng/L) levels in females; grey bands represent prediction intervals of median NT-proBNP.

We validated their associations with NT-proBNP in the overall population, and in males and females separately (online supplementary *Table S3*). After adjusting for these covariates in model 2, there was a strong and inverse association between WC and

Table 3 Associations of N-terminal pro-B-type natriuretic peptide with age and sex (combined population)

	Univariable			Model 1[a]			Model 2[b]		
	Sβ	R^2	*P*-value	Sβ	R^2	*P*-value	Sβ	R^2	*P*-value
Age	0.361	0.130	<0.001	–	–	–	–	–	–
Female sex	0.286	0.082	<0.001	0.320	0.231	<0.001	0.337	0.285	<0.001

HOMA-IR, homeostasis model assessment of insulin resistance; Sβ, standardized β coefficient.
[a]Age-adjusted associations.
[b]Associations after adjusting for age, history of myocardial infarction, hypertension, antihypertensive medication, insulin resistance (HOMA-IR), glomerular filtration rate and high-sensitive C-reactive protein.

NT-proBNP in females (S$\beta = -0.166$, $P < 0.001$) and a weaker inverse association in males (S$\beta = -0.074$, $P < 0.001$), with a significant difference between the sexes ($P_{interaction} < 0.001$). The associations of BMI and body weight with NT-proBNP levels in males and females exhibited an identical pattern (*Table 4*).

Association of abdominal obesity with NT-proBNP

Finally, we evaluated the relationship of NT-proBNP with increasing abdominal obesity using a multivariable model that adjusted for all covariates in model 2, but now also correcting for BMI. Increasing WC was associated with lower NT-proBNP levels only in females (S$\beta = -0.076$, $P = 0.014$), but not in males (S$\beta = -0.008$, $P = 0.790$) ($P_{interaction} < 0.001$) (*Figure 5*).

Grouping according to tertiles of age showed that there was a significant inverse association between abdominal obesity and NT-proBNP levels in females in the highest age tertile (mean age: 65 years). There was also a significant interaction between NT-proBNP and sex in this age tertile (online supplementary *Table S5A*). When women were grouped according to menopausal status, abdominal obesity was significantly associated with lower NT-proBNP levels in post-menopausal women with a menopausal status >2 years (mean age: 62 years) (online supplementary *Table S6A*).

Further adjustment for UAE, both as a continuous variable and as a categorical variable, did not affect any of the results significantly (online supplementary *Tables S4*, *S5B*, *S6B*).

Discussion

Principal findings

Using a large sample of individuals from the general population, we investigated the effect of sex on 'obesity-associated decrease' in NT-proBNP levels. Our results indicate that natriuretic peptide deficiency in obesity has a significant sex-associated component. Sex modified the inverse association of NT-proBNP with obesity such that this negative association was more pronounced among females compared with males. Importantly, abdominal obesity was associated with lower NT-proBNP levels in females but not in males. Sex also modified the direct association of NT-proBNP with age such that age-related increases in NT-proBNP were much greater among males compared with females. Our data therefore suggest that while interpreting NT-proBNP levels in clinical practice, particularly in obese individuals, sex and age should be considered.

NT-proBNP levels in obesity: impact of sex and age

Several studies, including ours, indicate that baseline NT-proBNP levels are lower in males compared with females.[7,8] Previous studies also report that circulating natriuretic peptides are lower in obese individuals compared to normal weight individuals[14,16,28–30]; however, sex differences in 'obesity-associated reduction' of NT-proBNP were not analysed in depth.

Table 4 Sex stratified associations of N-terminal pro-B-type natriuretic peptide with obesity parameters

	Model 1[a]						Interaction *P*-value	Model 2[b]						Interaction *P*-value
	Males			Females				Males			Females			
	Sβ	R^2	*P*-value	Sβ	R^2	*P*-value		Sβ	R^2	*P*-value	Sβ	R^2	*P*-value	
Waist circumference (SD)	−0.072	0.274	<0.001	−0.129	0.074	<0.001	<0.001	−0.074	0.341	<0.001	−0.166	0.120	<0.001	<0.001
Body mass index (SD)	−0.084	0.277	<0.001	−0.129	0.075	<0.001	0.001	−0.083	0.342	<0.001	−0.171	0.122	<0.001	0.001
Weight (SD)	−0.056	0.273	<0.001	−0.088	0.067	<0.001	0.040	−0.048	0.339	0.002	−0.117	0.112	<0.001	0.022

HOMA-IR, homeostasis model assessment of insulin resistance; R^2, adjusted R-squared; Sβ, standardized β coefficient; SD, standard deviation.
[a]Age-adjusted associations.
[b]Associations after adjusting for age, history of myocardial infarction, hypertension, antihypertensive medication, insulin resistance (HOMA-IR), glomerular filtration rate and high-sensitive C-reactive protein.

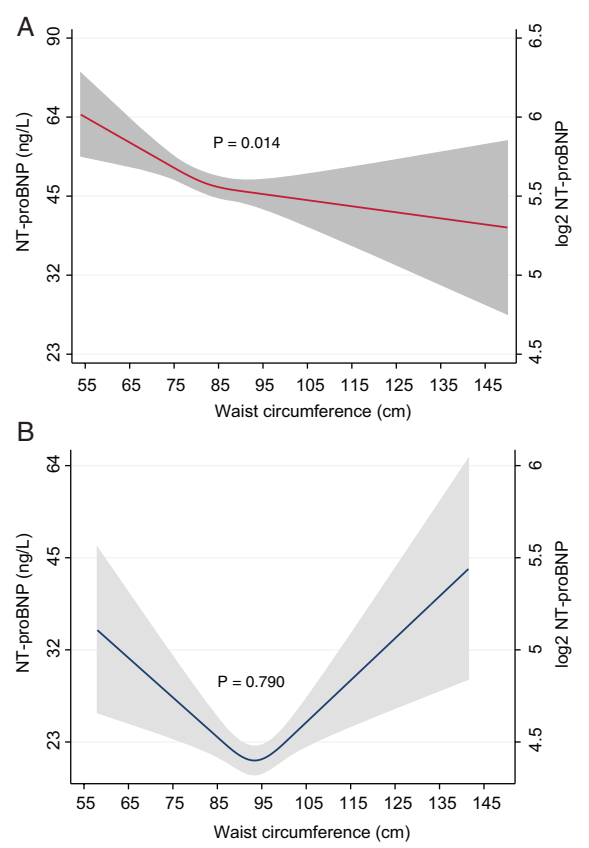

Figure 5 (*A*) Restricted cubic spline curves depicting associations of waist circumference with N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels after multivariable adjustment in females. Red lines represent plasma NT-proBNP (ng/L) levels in females; restricted cubic spline curves represent associations after adjusting for age, myocardial infarction, hypertension, antihypertensive drug usage, insulin resistance, glomerular filtration rate, C-reactive protein and body mass index. (*B*) Restricted cubic spline curves depicting associations of waist circumference with NT-proBNP levels after multivariable adjustment in males. Blue lines represent plasma NT-proBNP (ng/L) levels in males; restricted cubic spline curves represent associations after adjusting for age, myocardial infarction, hypertension, antihypertensive drug usage, insulin resistance, glomerular filtration rate, C-reactive protein and body mass index.

In our study, the relationship between obesity (i.e. increasing WC and BMI) and NT-proBNP in the general population was significantly modified by sex; in fact, after sex stratification, males surprisingly displayed *higher* NT-proBNP levels with increasing WC and BMI (*Figure 2B*, and online supplementary *Figure S2B*). Age, however, strongly confounded the relationship of these parameters with NT-proBNP.

We also demonstrate that lower NT-proBNP levels in individuals with higher body weight are better explained by sex than by obesity as males (on an average) weigh more than females, but also have lower median NT-proBNP levels. After stratifying according to sex, increasing body weight was not associated with NT-proBNP levels in females, while heavier males displayed slightly lower NT-proBNP levels; interestingly, variation of age was minimal among tertiles of weight in men.

Increasing age is associated with progressively higher NT-proBNP levels in the general population,[7,8] and our current study dissects that age-associated increase in NT-proBNP is significantly greater in males than in females. Furthermore, the relationship between age and higher NT-proBNP levels appears to be more uniform in males. In females, the positive association of age with NT-proBNP is remarkable from approximately 55–60 years (*Figure 3B*).

Age-associated increase in NT-proBNP could directly result from CV disorders (e.g. hypertension, MI) or as a consequence of comorbidities such as renal dysfunction. Other factors associated with NT-proBNP include insulin resistance, low-grade systemic inflammation and antihypertensive medication usage.[12,27] After adjusting for the above-mentioned factors including age, our study unveils that NT-proBNP levels decline with obesity, more in females than in males.

Mechanisms affecting NT-proBNP levels in obesity

It is currently hypothesized that natriuretic peptide (i.e. BNP and NT-proBNP) secretion is either inhibited by factors released by adipose tissue or actively metabolized by clearance receptors (e.g. NPR-C) in obese individuals.[28,30] Van Kimmenade *et al.*[31] explored these hypotheses and demonstrated that weight loss is associated with a concomitant increase in both BNP and NT-proBNP. As NPR-C activity is more specific for BNP and does not affect NT-proBNP clearance, this study illustrates that obesity suppresses their common production rather than increasing their clearance.[31]

Khan *et al.*[27] evaluated mechanisms underlying the relative natriuretic peptide deficiency associated with obesity using two large cohorts, and also performed analysis in males and females separately; however, no direct comparison was made between males and females. The study concluded that obesity may confer such effects partly through insulin resistance. We, therefore evaluated the effects of obesity on NT-proBNP levels after accounting for major CV risk factors including insulin resistance, and our results indicate that even after multivariable adjustment the inverse relationship of obesity with NT-proBNP is stronger in females than in males (*Table 4*).

Furthermore, we specifically evaluated the effects of abdominal obesity on NT-proBNP levels after accounting for general fat distribution (i.e. BMI), and demonstrate that abdominal obesity is associated with lower NT-proBNP levels in females, but not in males. An important mechanism through which abdominal obesity affects plasma concentrations of NT-proBNP could be through modulation of sex hormones, especially testosterone. According to data from randomized controlled trials, testosterone administration reduces NT-proBNP levels in both females[32] and males.[33] Interestingly, visceral fat has been recognized to increase circulating testosterone levels, particularly in females,[34–36] and this may explain why women with abdominal obesity have lower

NT-proBNP levels. However, this mechanism may not operate in males as visceral adiposity, unlike in females, is associated with decreased androgen levels.[35]

Our study also indicates that the effect of abdominal obesity on NT-proBNP levels appears to be more prominent in older females, particularly in post-menopausal women (online supplementary *Table S6*). Natriuretic peptides are cardioprotective and play an important role in sodium and water excretion, and in the reduction of blood pressure. Post-menopausal women have a higher risk of developing CV disorders, and lower natriuretic peptides associated with abdominal obesity could result in increased blood pressure and reduced cardioprotection, accelerating the development of unfavourable CV outcomes.[37,38]

Abdominal obesity may thus elicit a 'high-risk CV phenotype' in women by promoting a state of cardiac endocrine insufficiency resulting in reduced natriuretic peptide production by the heart. Further mechanistic studies are needed to verify this hypothesis.

Study strengths and limitations

The PREVEND study is a large, well-characterized community-based cohort with an almost 50–50 sex ratio and a relatively low mean age. Careful measurement and documentation of routine blood tests and anthropometric parameters were performed in all participants, and plasma NT-proBNP concentrations were measured using a reliable assay. Furthermore, in our statistical analyses, we accounted for non-linear associations of obesity with NT-proBNP levels using a regression analysis with a restricted cubic spline function.

We acknowledge certain limitations of our study. This is a post-hoc study and PREVEND cohort was enriched for increased UAE, but this overrepresentation was overcome using a statistical correction method. Although we excluded individuals with prevalent HF in our baseline cohort, it is possible that some of these individuals had prevalent HF with preserved ejection fraction or HF with mid-range ejection fraction, as such a distinction is made only in the more recent 2016 European Society of Cardiology guidelines.[39] The assay used in our study uses two polyclonal antibodies for detection of epitopes in NT-proBNP and has a functional sensitivity of <50 ng/L; more recently developed assays use monoclonal antibodies for detection—however, their analytical performance is comparable to the polyclonal ECLIA method. Although WC, BMI and body weight are regularly utilized markers of (abdominal) obesity, more accurate measures of fat distribution could be obtained using magnetic resonance imaging; however, such modalities may not be practical to be employed in large population studies. Another limitation is that our cohort is predominantly Caucasian and therefore our results should be validated in other ethnicities and population groups. Finally, our study is observational, therefore we are unable to draw conclusions regarding causality.

Concluding remarks

Firstly, we demonstrate that a large part of the 'obesity-associated reduction' in NT-proBNP levels in the general population is explained by sex. Secondly, age confounds the relationship of obesity with NT-proBNP, and age-associated increase in NT-proBNP is greater in men than in women. Finally, the inverse relationship between obesity and NT-proBNP levels is stronger in females compared to males, and abdominal obesity is associated with lower NT-proBNP levels in females, but not in males. Further studies are needed to understand the mechanisms through which abdominal obesity reduces NT-proBNP levels in women.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Methods S1. Supplementary methods.

Table S1. Baseline characteristics by tertiles of body mass index (BMI) in the combined population (*A*) and in males and females (*B*).

Table S2. Baseline characteristics by tertiles of body weight in the combined population (*A*) and in males and females (*B*).

Table S3. Association of NT-proBNP with cardiovascular risk factors.

Table S4. Relationship of NT-proBNP with abdominal obesity after adjusting for urinary albumin excretion.

Table S5. Association of abdominal obesity with NT-proBNP in males and females according to age categories before (*A*) and after (*B*) adjusting for urinary albumin excretion.

Table S6. Association of abdominal obesity with NT-proBNP levels: impact of menopausal status before (*A*) and after (*B*) adjusting for urinary albumin excretion.

Figure S1. PREVEND flow diagram.

Figure S2. (*A*) Association of body mass index with NT-proBNP levels in the overall population using kernel-weighted local polynomial smoothing graphic modelling. (*B*) Association of body mass index with NT-proBNP levels in males and females.

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