Plasma Proneurotensin and Incidence of Diabetes, Cardiovascular Disease, Breast Cancer, and Mortality

Olle Melander, MD, PhD
Alan S. Maisel, MD
Peter Almgren, MSc
Jonas Manjer, MD, PhD
Mattias Belting, MD, PhD
Bo Hedblad, MD, PhD
Gunnar Engström, MD, PhD
Ute Kilger, PhD
Peter Nilsson, MD, PhD
Andreas Bergmann, PhD
Marju Orho-Melander, PhD

Context Neurotensin regulates both satiety and breast cancer growth in the experimental setting, but little is known about its role in the development of breast cancer or cardiometabolic disease in humans.

Objective To test if fasting plasma concentration of a stable 117-amino acid fragment from the neurotensin precursor hormone proneurotensin is associated with development of diabetes mellitus, cardiovascular disease, breast cancer, and mortality.

Design, Setting, and Participants Proneurotensin was measured in plasma from 4632 fasting participants of the population-based Malmö Diet and Cancer Study baseline examination 1991-1994. Multivariate Cox proportional hazards models were used to relate baseline proneurotensin to first events and death during long-term follow-up until January 2009, with median follow-up ranging from 13.2 to 15.7 years depending on the disease.

Main Outcome Measures Incident diabetes mellitus, cardiovascular disease, breast cancer, and mortality.

Results Overall, proneurotensin (hazard ratio [HR] per SD increment of log-transformed proneurotensin) was related to risk of incident diabetes (142 events; HR, 1.28; 95% CI, 1.09-1.50; $P=.003$), cardiovascular disease (519 events; HR, 1.17; 95% CI, 1.07-1.27; $P<.001$), and cardiovascular mortality (174 events; HR, 1.29; 95% CI, 1.12-1.49; $P=.001$) with a significant interaction between proneurotensin and sex ($P<.001$) on risk of cardiovascular disease. Exclusively in women, proneurotensin was related to incident diabetes (74 events; HR, 1.41; 95% CI, 1.12-1.77; $P=.003$), cardiovascular disease (224 events; HR, 1.33; 95% CI, 1.17-1.51; $P<.001$), breast cancer (123 events; HR, 1.44; 95% CI, 1.21-1.71; $P<.001$), total mortality (285 events; HR, 1.13; 95% CI, 1.01-1.27; $P=.03$), and cardiovascular mortality (75 events; HR, 1.50; 95% CI, 1.20-1.87; $P<.001$).

Conclusion Fasting proneurotensin was significantly associated with the development of diabetes, cardiovascular disease, breast cancer, and with total and cardiovascular mortality.

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Author Affiliations: Department of Clinical Sciences, Lund University, Malmö, Sweden (Drs Melander, Almgren, Manjer, Hedblad, Engström, Nilsson, and Orho-Melander); and Section of Oncology, Lund, Sweden (Dr Belting); Center of Emergency Medicine (Drs Melander, Hedblad, and Nilsson) and Departments of Surgery (Dr Manjer) and Oncology (Dr Belting), Skåne University Hospital, Lund and Malmö, Sweden; Veterans Affairs San Diego Healthcare Systems, San Diego, California (Dr Maisel); SphingoTec GmbH, Hohen Neuendorf, Germany (Drs Kilger and Bergmann); and Waltraut Bergmann Foundation, Hohen Neuendorf, Germany (Dr Bergmann).
Corresponding Author: Olle Melander, MD, PhD, Department of Clinical Sciences, Malmö, CRC, Ent 72, Bldg 91, Level 12, Skåne University Hospital, SE 205 02 Malmö, Sweden (olle.melander@med.lu.se).

BARIATRIC SURGERY OF PATIENTS with obesity has been associated with marked reduction of the incidence of diabetes, cardiovascular disease and mortality, and, in women specifically, with reduction of the incidence of cancer.[1-4] However, the causes of obesity-associated morbidity and mortality are poorly understood. Neurotensin is a 13-amino acid peptide primarily expressed in the central nervous system and gastrointestinal tract.[5-7] Neurotensin binds to 3 different receptors: neurotensin receptor 1 and 2 (Ntsr1 and Ntsr2), which are G-protein coupled receptors, and neurotensin receptor 3 (Ntsr3), which is non–G-protein coupled and also known as sortilin-1 (SORT1).[8,9] The peripheral secretion of neurotensin is stimulated by food intake, especially by fat, and is known to regulate gastrointestinal motility and

pancreatic and biliary secretion.[10] Both central (intracerebroventricular) and peripheral (intraperitoneal) injection of neurotensin acutely reduces food intake in rats, an effect mediated through Ntsr1.[11,12]

In obese as compared with normal-weight humans, postprandial plasma neurotensin concentration was reduced following a liquid fatty meal[13] and increased after gastric bypass (Roux-en-Y) treatment,[14,15] suggesting that regulation of neurotensin secretion is disturbed in human obesity. However, no study has investigated if and how neurotensin is related to measures of obesity or to obesity-associated diseases in the general population.

Neurotensin has trophic effects both on normal and neoplastic tissue and neurotensin and Ntsr1 have been suggested to be prognostic tumor biomarkers.[16,17] Neurotensin and Ntsr1 expression is common in human malignant ductal breast cancer tumors, and in mice xenografted with a malignant human breast cancer cell line, pharmacological blockade or RNA silencing of Ntsr1 reduces tumor growth.[18,19] In addition, genetic variation of 1 of the 3 receptors for neurotensin, ie, Ntsr3 (SORT1), is linked to development of coronary artery disease in humans, an effect mediated by elevated levels of low-density lipoprotein cholesterol (LDL-C).[20,21]

We hypothesized that variations of the neurotensin system may contribute to development of common diseases associated with elevated body mass index (BMI). Because mature neurotensin is unstable both in vitro and in vivo, we measured a stable 117-amino acid fragment from the *N*-terminal part of the pre-proneurotensin/neuromedin precursor hormone, referred to as proneurotensin, which is produced in stoichiometric amounts relative to the mature neurotensin.[22] Certain tissues partially produce large peptides composed of proneurotensin connected to the peptide sequences corresponding to the mature hormones (large neurotensin and large neuromedin).[23] These large hormones have been shown to weakly bind and activate Ntsr1[23]; however, it is unknown if proneurotensin has biological activity. In this study, we explored whether fasting concentration of proneurotensin is associated with future risk of diabetes mellitus, cardiovascular disease, and breast cancer, as well as with death.

METHODS

Study Population

The Malmö Diet and Cancer (MDC) study is a population-based, prospective epidemiologic cohort of 28 449 men (born 1923-1945) and women (born 1923-1950) from Malmö, Sweden, who underwent baseline examinations between 1991 and 1996.[24] From this cohort, 6103 individuals were randomly selected to participate in the MDC Cardiovascular Cohort (MDC-CC), which was designed to investigate the epidemiology of carotid artery disease between 1991 and 1994.[25] Fasting plasma samples were available for analysis of proneurotensin in 4632 participants in the MDC-CC. The 1471 excluded participants (due to lack of plasma sample) were slightly younger, but did not otherwise differ in terms of sex, smoking, diabetes, hypertension status, BMI, or plasma lipids as detailed in the eAppendix (available at http://www.jama.com).

Of the 4632 participants in whom proneurotensin was measured, those with prevalent disease prior to the baseline examination were excluded from the analyses of the main outcomes, as well as those without complete data on covariates. The baseline examination procedure has been described previously (eAppendix).[26-28] Proneurotensin was measured in stored fasting plasma specimens that were frozen to –80°C immediately at the MDC-CC baseline examination using a recent chemiluminometric sandwich immunoassay to detect a proneurotensin precursor fragment (proneurotensin 1–117) (eAppendix).[22]

Following procedures (eAppendix) previously described,[26-30] we used Swedish national and local registers to retrieve incident cases of diabetes mellitus, cardiovascular disease (myocardial infarction and stroke), breast cancer, and all-cause and cause-specific mortality during more than 12 years of follow-up. Follow-up extended to January 1, 2009, for all end points except for new-onset diabetes, for which follow-up extended to June 30, 2006.

All participants gave written informed consent and the study was approved by the ethical committee at Lund University, Lund, Sweden.

Statistics

We used multivariate Cox proportional hazards models to test the relationship between fasting plasma concentration of proneurotensin and each of the outcomes. Because we had primary hypotheses of association between proneurotensin and each of the outcomes, we did not adjust for multiple comparisons. In analyses of incident diabetes, we adjusted for age, sex, use of antihypertensive medication, systolic blood pressure, BMI, waist circumference, prevalent cardiovascular disease, current smoking, and fasting concentrations of glucose, high-density lipoprotein cholesterol (HDL-C), LDL-C, triglycerides, and insulin (diabetes risk factors).

In analyses of incident cardiovascular disease, all-cause mortality and cardiovascular mortality, we adjusted for age, sex, use of antihypertensive medication, systolic blood pressure, BMI, current smoking, diabetes mellitus, and fasting concentrations of HDL-C and LDL-C (cardiovascular disease risk factors). In analyses of incident breast cancer (women only), we adjusted for age, use of antihypertensive medication, use of hormone therapy, ever use of oral contraceptives, educational level, age at menarche, number of children, menopausal status, systolic blood pressure, BMI, diabetes mellitus, current smoking, prevalent cardiovascular disease, heredity for cancer, and fasting concentrations of HDL, LDL and insulin (breast cancer risk factors). All analyses were analyzed and checked for multicollinearity between covariates but no significant multicollinearity was found in any analyses.

Fasting plasma concentration of proneurotensin was skewed to the right and therefore transformed with the natural logarithm and thereafter normalized, and hazard ratios (HRs) were expressed per 1 (SD) increment of log-transformed proneurotensin in the Cox proportional hazards models. In addition, proneurotensin was divided into quartiles. Quartile 1 (lowest values of proneurotensin) was defined as the reference standard and quartiles 2 to 4 were compared with the reference quartile in the Cox proportional hazards models.

All analyses were performed with Stata statistical software version 11. A 2-sided *P* value of less than .05 was considered statistically significant.

RESULTS

Cross-sectional Relationship Between Cardiometabolic Risk Factors and Proneurotensin

The baseline characteristics are shown in TABLE 1 and in eTable 1 and eTable 2. Women had significantly higher proneurotensin (median [interquartile range {IQR}] than men: 109 pmol/L [79-150] vs 99 pmol/L [71-144]; $P<.001$). The relationship between proneurotensin and cardiometabolic risk factors was weak, with the strongest correlation being that with fasting insulin concentration in both sexes (eTable 3). In a linear regression model with backward elimination and a retention $P<.10$, significant independent determinants of proneurotensin were smoking and fasting concentrations of insulin, glucose, and HDL (all positive) in women, and smoking and fasting concentrations of insulin and HDL (positively related) and age and LDL (negatively related) in men (TABLE 2).

Proneurotensin and Risk of Diabetes Mellitus

Among 3704 participants free from diabetes mellitus at baseline (1484 men and 2220 women), 142 (68 men and 74 women) developed new-onset diabetes mellitus during a median (IQR) follow-up time of 13.2 years (12.6-13.7) with an event rate of 30.2 per 10 000 person-years. Each SD increase of baseline proneurotensin was associated with a multivariate-adjusted HR of 1.28 (95% CI, 1.09-1.50; $P=.003$) for the risk of new-onset diabetes in the total study population, whereas the HR was 1.41 (95% CI, 1.12-1.77; $P=.003$) in women, and not significantly elevated in men (HR, 1.21; 95% CI, 0.96-1.53; $P=.10$). There was no significant interaction between sex and proneurotensin regarding the association with new-onset diabetes ($P=.37$).

Table 1. Clinical Characteristics of the Study Population in Analyses of Incident Cardiovascular Disease, Cardiovascular Mortality, and Total Mortality

	Women (n = 2559)	Men (n = 1802)
Age, mean (SD), y	57.6 (5.9)	57.8 (6.0)
Systolic blood pressure, mean (SD), mm Hg	141 (19)	144 (19)
Diastolic blood pressure, mean (SD), mm Hg	85.7 (9.1)	89.0 (9.7)
Antihypertensive therapy, No. (%)	386 (15.1)	292 (16.2)
Diabetes mellitus, No. (%)	163 (6.4)	206 (11.4)
Fasting blood glucose, mean (SD), mmol/L	5.0 (1.2)	5.4 (1.5)
Fasting insulin concentration, (median IQR), μIU/mL	6.0 (4.0-9.0)	7.0 (5.0-10)
BMI, mean (SD)[a]	25.5 (4.2)	26.1 (3.4)
Waist circumference, mean (SD), cm	77.0 (10.2)	92.9 (9.9)
LDL-C, mean (SD), mmol/L	4.2 (1.0)	4.1 (0.89)
HDL-C, mean (SD), mmol/L	1.5 (0.37)	1.2 (0.30)
Triglycerides, mean (SD), mmol/L	1.2 (0.58)	1.4 (0.68)
Current smokers, No. (%)	653 (25.5)	497 (27.6)

Abbreviations: BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
SI conversion factors: To convert blood glucose to mg/dL, divide by 0.0555; insulin concentration to pmol/L, multiply by 6.945; HDL-C to mg/dL, divide by 0.0259; LDL-C to mg/dL, divide by 0.0259; triglycerides to mg/dL, divide by 0.0113.
[a] BMI calculated as weight in kilograms divided by height in meters squared.

Table 2. Independent Significant Determinants of Fasting Plasma Concentration of Proneurotensin in Women and Men From a Linear Regression Model With Backward Elimination[a]

	Women (n = 2559)		Men (n = 1802)	
Independent Proneurotensin Determinants	β Coefficient (95% CI)[b]	*P* Value	β Coefficient (95% CI)[b]	*P* Value
Insulin[c]	0.15 (0.10 to 0.20)	<.001	0.15 (0.09 to 0.20)	<.001
High-density lipoprotein	0.05 (0.00 to 0.09)	.04	0.05 (0.00 to 0.10)	.05
Smoking	0.16 (0.07 to 0.25)	<.001	0.13 (0.03 to 0.24)	.01
Glucose	0.07 (0.03 to 0.11)	.001		
Age			–0.05 (–0.10 to –0.01)	.02
Low-density lipoprotein			–0.05 (–0.10 to –0.01)	.03

[a] The following variables were entered in the linear regression model with backward elimination at $P<.10$: age, antihypertensive treatment, systolic blood pressure, diastolic blood pressure, current smoking, waist circumference, body mass index, and fasting concentrations of glucose, insulin, triglycerides, high-density lipoprotein, and low-density lipoprotein.
[b] The β is expressed as the increment of the log-transformed standardized values of proneurotensin per increment of standardized values (or presence of dichotomized risk factor) of the risk factor in question.
[c] Insulin was log-transformed before being standardized.

Among 1950 women free from impaired fasting glucose at baseline (fasting whole blood glucose, <97 mg/dL [<5.4 mmol/L]), 38 women developed diabetes during follow-up with an event rate of 15.0 per 10 000 person-years, and each SD increase of baseline proneurotensin was associated with a multivariate-adjusted HR

Table 3. Event Rates and Multivariate Adjusted Cox Proportional Hazards Models for Baseline Proneurotensin vs Incidence of Cardiovascular Disease, Breast Cancer, All-Cause Mortality, and Cardiovascular Mortality

		Quartiles					
	Overall	1	2	3	4	*P* Value[a]	*P* for trend
Cardiovascular disease[b]							
All participants, No./events, No.	4361/519	1091/118	1090/113	1092/143	1088/145		
Events/10 000 person-years	82.5	74.8	71.0	91.2	93.2		
Proneurotensin, median (range), pmol/L[c]	105 (3.3-1155)	60.2 (3.3-75.7)	89.3 (75.8-105)	123 (105-148)	190 (148-1155)		
HR (95% CI)[d]	1.17 (1.07-1.27)	1 [Reference]	1.09 (0.84-1.41)	1.39 (1.09-1.78)	1.37 (1.07-1.75)	<.001	.003
Women, No./events, No.	2559/224	640/44	641/39	639/68	639/73		
Events/10 000 person-years	59.2	46.4	40.5	72.3	78.1		
Proneurotensin, median (range), pmol/L[c]	109 (5.1-1155)	62.4 (5.1-78.6)	92.1 (78.6-109)	125 (109-150)	194 (150-1155)		
HR (95% CI)[d]	1.33 (1.17-1.51)	1 [Reference]	0.91 (0.59-1.40)	1.58 (1.08-2.32)	1.65 (1.13-2.41)	<.001	.001
Men, No./events, No.	1802/295	451/67	450/79	451/74	450/75		
Events/10 000 person-years	118	106	125	119	121		
Proneurotensin, median (range), pmol/L[c]	98.9 (3.3-1057)	58.0 (3.3-70.8)	85.9 (71.0-98.8)	118 (98.9-144)	186 (144-1057)		
HR (95% CI)[d]	1.06 (0.95-1.19)	1 [Reference]	1.27 (0.92-1.76)	1.27 (0.91-1.77)	1.22 (0.88-1.70)	.31	.27
All-cause mortality[b]							
All participants, No./events, No.	4361/603	1091/141	1090/135	1092/151	1088/176		
Events/10 000 person-years	92.2	86.6	82.1	92.2	108		
HR (95% CI)[d]	1.08 (1.00-1.17)	1 [Reference]	1.05 (0.82-1.32)	1.17 (0.93-1.48)	1.30 (1.04-1.63)	.05	.01
Women, No./events, No.	2559/285	640/62	641/65	639/67	639/91		
Events/10 000 person-years	73.3	63.9	66.5	68.8	94.1		
HR (95% CI)[d]	1.13 (1.01-1.27)	1 [Reference]	1.08 (0.76-1.52)	1.07 (0.76-1.52)	1.43 (1.03-1.97)	.03	.04
Men, No./events, No.	1802/318	451/75	450/73	451/85	450/85		
Events/10 000 person-years	120	114	110	129	128		
HR (95% CI)[d]	1.04 (0.93-1.16)	1 [Reference]	1.01 (0.73-1.40)	1.25 (0.91-1.71)	1.15 (0.84-1.58)	.47	.21
Cardiovascular mortality[b]							
All participants, No./events, No.	4361/174	1091/37	1090/31	1092/46	1088/60		
Events/10 000 person-years	26.6	22.7	18.9	28.1	36.8		
HR (95% CI)[d]	1.29 (1.12-1.49)	1 [Reference]	0.95 (0.59-1.53)	1.40 (0.91-2.17)	1.73 (1.14-2.61)	.001	.003
Women, No./events, No.	2559/75	640/13	641/13	639/20	639/29		
Events/10 000 person-years	19.3	13.4	13.3	20.6	30.0		
HR (95% CI)[d]	1.50 (1.20-1.87)	1 [Reference]	1.02 (0.47-2.21)	1.53 (0.76-3.09)	2.18 (1.13-4.20)	<.001	.008
Men, No./events, No.	1802/99	451/21	450/22	451/26	450/30		
Events/10 000 person-years	37.3	31.8	33.0	39.3	45.2		
HR (95% CI)[d]	1.16 (0.96-1.41)	1 [Reference]	1.07 (0.59-1.96)	1.36 (0.77-2.43)	1.44 (0.82-2.53)	.13	.14
Breast cancer[e]							
Women, No./events, No.	1929/123	483/20	483/25	481/32	482/46		
Events/10 000 person-years	43.2	28.0	34.7	44.8	65.7		
Proneurotensin, median (range), pmol/L[c]	108 (5.1-1132)	62.1 (5.1-77.5)	91.1 (77.6-108)	125 (108-150)	194 (150-1132)		
HR (95% CI)[d]	1.44 (1.21-1.71)	1 [Reference]	1.32 (0.73-2.38)	1.79 (1.02-3.14)	2.44 (1.44-4.15)	<.001	<.001

Abbreviations: BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; LDL-C, low-density lipoprotein cholesterol.

[a] *P* values are for continuous analyses of association between proneurotensin and outcomes.

[b] Analyses of incident cardiovascular disease, all-cause mortality, and cardiovascular mortality were adjusted for age, sex, use of antihypertensive medication, systolic blood pressure, BMI, current smoking, diabetes mellitus, and fasting HDL-C and LDL-C.

[c] Because participants included and the respective proneurotensin values (fasting plasma concentration) are the same for cardiovascular disease, all-cause mortality, and cardiovascular mortality, proneurotensin values are only shown for cardiovascular disease and breast cancer.

[d] HRs (95% CIs) are expressed per 1-SD increment of log-transformed proneurotensin (in analyses of all participants, all men, and all women).

[e] Analysis of incident breast cancer in women was adjusted for age, use of antihypertensive medication, hormone therapy, ever use of oral contraceptives, educational level, age at menarche, number of children, menopausal status, systolic blood pressure, BMI, diabetes mellitus, current smoking, prevalent cardiovascular disease, heredity for cancer, and fasting concentrations of HDL-C, LDL-C, and insulin.

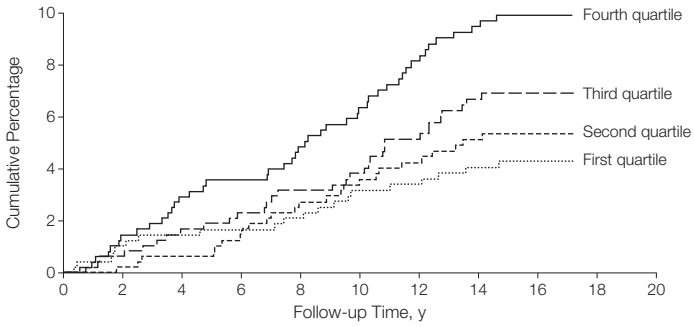
of 1.47 (1.08-2.00; $P=.01$) for incident diabetes.

Proneurotensin and Risk of Cardiovascular Disease, Cardiovascular Mortality, and All-Cause Mortality

Among 4361 participants without cardiovascular disease prior to the baseline examination (1802 men and 2559 women), 519 developed a first cardiovascular disease event during a median (IQR) follow-up time of 15.6 years (14.9-16.2), with an event rate of 82.5 per 10 000 person-years. After full adjustment, each SD increase of proneurotensin was associated with an HR of 1.17 (95% CI, 1.07-1.27) for risk of incident cardiovascular disease (TABLE 3). There was a significant interaction between proneurotensin and female sex on incidence of cardiovascular disease ($P<.001$) and sex-stratified analyses revealed that each SD increase of baseline proneurotensin was associated with a hazard ratio of 1.33 (95% CI, 1.17-1.51) and top vs bottom quartile of proneurotensin with a hazard ratio of 1.65 (95% CI, 1.13-2.41) for risk of cardiovascular disease in women, whereas there was no significant relationship among men (Table 3).

Each SD increment of proneurotensin was associated with a hazard ratio of 1.08 (95% CI, 1.00-1.17) for risk of all-cause mortality in the total population and a hazard ratio of 1.13 (95% CI, 1.01-1.27) for risk of all-cause mortality among women, with no increased risk related to proneurotensin in men (Table 3). The excess risk of death associated with proneurotensin in women appeared to be mainly accounted for by cardiovascular deaths with a hazard ratio of 1.50 (95% CI, 1.20-1.87) per SD increment of proneurotensin and 2.18 (95% CI, 1.13-4.20) in women belonging to the top as compared with the bottom quartile of proneurotensin (Table 3). There was no significant interaction between sex and proneurotensin regarding the association with all-cause ($P=.26$) and cardiovascular mortality ($P=.08$).

Figure. Cumulative Breast Cancer Event-Free Survival During Follow-up



No. at risk

First quartile	483	476	472	463	454	445	434	426	151
Second quartile	483	482	476	470	459	448	436	420	151
Third quartile	481	477	468	460	451	440	431	414	168
Fourth quartile	482	472	463	455	440	426	416	398	187

Kaplan-Meier plot shows 1 minus cumulative breast cancer event-free survival during follow-up in quartiles: first (lowest values) to fourth quartile of the baseline fasting plasma concentration of proneurotensin. Median (range) concentrations of the quartiles 1 to 4 are shown in Table 3. The numbers at risk are shown at 2-year intervals.

Proneurotensin and Breast Cancer in Women

During a median follow-up time of 15.7 years (IQR, 15.1-16.2) of 1929 women without cancer prior to the baseline examination, there were 123 incident cases of breast cancer with an event rate of 43.2 per 10 000 person-years. The cumulative incidence of breast cancer in quartiles of baseline fasting plasma concentration of proneurotensin is depicted in the FIGURE. After adjustment for breast cancer risk factors, each SD increase of proneurotensin was associated with an HR of 1.44 (95% CI, 1.21-1.71) for the risk of future breast cancer, and the top vs bottom quartiles of proneurotensin were associated with an HR of 2.44 (95% CI, 1.44-4.15) for risk of breast cancer (Table 3). In a model including a more limited set of breast cancer risk factors (age, use of hormone therapy, ever use of oral contraceptives, educational level, age at menarche, number of children, menopausal status, BMI, current smoking, and heredity for cancer), each SD increase of proneurotensin was associated with an HR of 1.42 (95% CI, 1.19-1.67; $P<.001$) for risk of breast cancer.

COMMENT

To our knowledge, this is the first epidemiological study on fasting concentration of proneurotensin, a stable *N*-terminal fragment of the precursor of the satiety hormone neurotensin, in relation to risk of future disease. We show that proneurotensin is associated with the development of diabetes mellitus, cardiovascular disease, total mortality, cardiovascular mortality, and breast cancer in women during long-term follow-up.

The relationship between proneurotensin and morbidity and mortality was only significant in women. It has been repeatedly shown that estradiol up-regulates expression of neurotensin.[18,31,32] Thus, it can be speculated that the higher proneurotensin observed in women than in men, and the fact that the association between proneurotensin and adverse outcomes was only significant in women, is partially explained by higher lifetime exposure to estrogen in women than in men. It should be noted that our study population included more women than men and that the interaction between proneurotensin and sex was only significant for the endpoint of incident cardiovascular dis-

ease. Thus, we cannot exclude that there may exist an association between proneurotensin and adverse outcomes also in men.

The elevation of proneurotensin several years before onset of disease indicates that proneurotensin is a marker of underlying disease susceptibility rather than being an expression of subclinical disease. As an observational study, our results do not prove any causation between proneurotensin and cardiometabolic disease and breast cancer. Two limitations of our study to consider when interpreting the results are that we did not correct for multiple comparisons and that we lack a replication cohort. Our results warrant replication in other prospective population-based studies and should encourage further research aimed at testing whether targeting the neurotensin system may have advantageous effects in preventing these common diseases in animal models and ultimately in humans.

As a satiety hormone, one may intuitively expect that high levels of proneurotensin would be related to less overeating and thus less obesity, diabetes, and cardiovascular disease. In contrast, high proneurotensin was persistently associated with increased risk of morbidity and mortality in women. The cause of this relationship is unclear. The key metabolic actions of neurotensin include digestion and metabolism of fat.[8,10] One can speculate that high plasma proneurotensin in the fasting state may be a result of compensatory increase in secretion of neurotensin due to resistance to the actions of neurotensin at the level of either its receptors or downstream of them, ie, neurotensin resistance.

Whatever the cause of high proneurotensin, experimental studies demonstrating increased expression of neurotensin and Nstr1 in breast tumors and reduced tumor growth after pharmacological blockade or RNA silencing of Ntsr1[18,19] lend support to a direct, mechanistic relationship between high-fasting proneurotensin and breast cancer development. Whereas the links between neurotensin and breast cancer are likely to be mediated through the Ntsr1, the major candidate receptor linking neurotensin to cardiovascular disease and diabetes is the Ntsr3 (SORT1), a protein that sorts various luminal proteins from the *trans*-Golgi. We and others identified genetic variation of the Ntsr3 as one of the strongest common susceptibility genes for coronary artery disease in the genome, an effect mediated through elevated levels of LDL-C.[20,21] In addition, Ntsr3 has been suggested to be an insulin-sensitive regulator of the key glucose transporter in muscle and adipose tissue, ie, glucose transporter 4 (GLUT4),[33] suggesting a role of the neurotensin system not only in metabolism of LDL-C and coronary artery disease but also in insulin resistance and diabetes development. In fact, fasting insulin concentration was one of the strongest correlates of proneurotensin.

Thus, it could be expected that insulin-resistant women, such as those with polycystic ovary syndrome, would drive the association with diabetes and cardiovascular disease in women. However, we had no data on polycystic ovary syndrome, however, the relationship between proneurotensin and cardiometabolic diseases was independent from both fasting insulin concentration and LDL-C. Thus, the mechanisms behind the relationship between high proneurotensin and cardiometabolic diseases remain to be identified.

In conclusion, fasting proneurotensin was significantly associated with development of diabetes, cardiovascular disease, breast cancer, and with total and cardiovascular mortality. The relationships between proneurotensin and all end points were significant in women but not in men; however, because there was only significant interaction between sex and proneurotensin for the outcome of incident cardiovascular disease, it remains to be shown whether the association between proneurotensin and adverse outcome is specific for women.

Author Contributions: Dr Melander had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Melander, Bergmann, Orho-Melander.
Acquisition of data: Melander, Manjer, Hedblad, Bergmann, Orho-Melander.
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Online-Only Material: eAppendix, eTables 1 to 3, and eReferences are available at http://www.jama.com.

REFERENCES

1. Sjöström CD, Lissner L, Wedel H, Sjöström L. Reduction in incidence of diabetes, hypertension and lipid disturbances after intentional weight loss induced by bariatric surgery: the SOS Intervention Study. *Obes Res*. 1999;7(5):477-484.
2. Sjöström L, Gummesson A, Sjöström CD, et al; Swedish Obese Subjects Study. Effects of bariatric surgery on cancer incidence in obese patients in Sweden (Swedish Obese Subjects Study): a prospective, controlled intervention trial. *Lancet Oncol*. 2009;10(7):653-662.
3. Sjöström L, Narbro K, Sjöström CD, et al; Swedish

Obese Subjects Study. Effects of bariatric surgery on mortality in Swedish obese subjects. *N Engl J Med*. 2007;357(8):741-752.
4. Sjöström L, Peltonen M, Jacobson P, et al. Bariatric surgery and long-term cardiovascular events. *JAMA*. 2012;307(1):56-65.
5. Carraway R, Leeman SE. The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalami. *J Biol Chem*. 1973;248(19):6854-6861.
6. Kitabgi P, Carraway R, Leeman SE. Isolation of a tridecapeptide from bovine intestinal tissue and its partial characterization as neurotensin. *J Biol Chem*. 1976; 251(22):7053-7058.
7. Minamino N, Kangawa K, Matsuo H. Neuromedin N: a novel neurotensin-like peptide identified in porcine spinal cord. *Biochem Biophys Res Commun*. 1984;122(2):542-549.
8. Kalafatakis K, Triantafyllou K. Contribution of neurotensin in the immune and neuroendocrine modulation of normal and abnormal enteric function. *Regul Pept*. 2011;170(1-3):7-17.
9. Vincent JP, Mazella J, Kitabgi P. Neurotensin and neurotensin receptors. *Trends Pharmacol Sci*. 1999; 20(7):302-309.
10. Kitabgi P. Prohormone convertases differentially process pro-neurotensin/neuromedin N in tissues and cell lines. *J Mol Med (Berl)*. 2006;84(8):628-634.
11. Cooke JH, Patterson M, Patel SR, et al. Peripheral and central administration of xenin and neurotensin suppress food intake in rodents. *Obesity (Silver Spring)*. 2009;17(6):1135-1143.
12. Kim ER, Mizuno TM. Role of neurotensin receptor 1 in the regulation of food intake by neuromedins and neuromedin-related peptides. *Neurosci Lett*. 2010; 468(1):64-67.
13. Wisén O, Björvell H, Cantor P, Johansson C, Theodorsson E. Plasma concentrations of regulatory peptides in obesity following modified sham feeding (MSF) and a liquid test meal. *Regul Pept*. 1992; 39(1):43-54.
14. Christ-Crain M, Stoeckli R, Ernst A, et al. Effect of gastric bypass and gastric banding on proneurotensin levels in morbidly obese patients. *J Clin Endocrinol Metab*. 2006;91(9):3544-3547.
15. Holdstock C, Zethelius B, Sundbom M, Karlsson FA, Edén Engström B. Postprandial changes in gut regulatory peptides in gastric bypass patients. *Int J Obes (Lond)*. 2008;32(11):1640-1646.
16. Dupouy S, Mourra N, Doan VK, Gompel A, Alifano M, Forgez P. The potential use of the neurotensin high affinity receptor 1 as a biomarker for cancer progression and as a component of personalized medicine in selective cancers. *Biochimie*. 2011;93(9):1369-1378.
17. Evers BM. Neurotensin and growth of normal and neoplastic tissues. *Peptides*. 2006;27(10):2424-2433.
18. Dupouy S, Viardot-Foucault V, Alifano M, et al. The neurotensin receptor-1 pathway contributes to human ductal breast cancer progression. *PLoS One*. 2009;4(1):e4223.
19. Souazé F, Dupouy S, Viardot-Foucault V, et al. Expression of neurotensin and NT1 receptor in human breast cancer: a potential role in tumor progression. *Cancer Res*. 2006;66(12):6243-6249.
20. Kathiresan S, Voight BF, Purcell S, et al; Myocardial Infarction Genetics Consortium; Wellcome Trust Case Control Consortium. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. *Nat Genet*. 2009;41(3):334-341.
21. Musunuru K, Strong A, Frank-Kamenetsky M, et al. From noncoding variant to phenotype via SORT1 at the 1p13 cholesterol locus. *Nature*. 2010;466(7307): 714-719.
22. Ernst A, Hellmich S, Bergmann A. Proneurotensin 1-117, a stable neurotensin precursor fragment identified in human circulation. *Peptides*. 2006; 27(7):1787-1793.
23. Friry C, Feliciangeli S, Richard F, Kitabgi P, Rovere C. Production of recombinant large proneurotensin/neuromedin N-derived peptides and characterization of their binding and biological activity. *Biochem Biophys Res Commun*. 2002;290(4):1161-1168.
24. Minisymposium: The Malmö Diet and Cancer Study: Design, biological bank and biomarker programme, 23 October 1991, Malmo, Sweden. *J Intern Med*. 1993;233(1):39-79.
25. Persson M, Berglund G, Nelson JJ, Hedblad B. Lp-PLA2 activity and mass are associated with increased incidence of ischemic stroke: a population-based cohort study from Malmö, Sweden. *Atherosclerosis*. 2008;200(1):191-198.
26. Belting M, Almgren P, Manjer J, et al. Vasoactive peptides with angiogenesis-regulating activity predict cancer risk in males. *Cancer Epidemiol Biomarkers Prev*. 2012;21(3):513-522.
27. Enhörning S, Wang TJ, Nilsson PM, et al. Plasma copeptin and the risk of diabetes mellitus. *Circulation*. 2010;121(19):2102-2108.
28. Melander O, Newton-Cheh C, Almgren P, et al. Novel and conventional biomarkers for prediction of incident cardiovascular events in the community. *JAMA*. 2009;302(1):49-57.
29. Rosvall M, Janzon L, Berglund G, Engström G, Hedblad B. Incident coronary events and case fatality in relation to common carotid intima-media thickness. *J Intern Med*. 2005;257(5):430-437.
30. Rosvall M, Janzon L, Berglund G, Engström G, Hedblad B. Incidence of stroke is related to carotid IMT even in the absence of plaque. *Atherosclerosis*. 2005; 179(2):325-331.
31. Alexander MJ. Estrogen-regulated synthesis of neurotensin in neurosecretory cells of the hypothalamic arcuate nucleus in the female rat. *Endocrinology*. 1993;133(4):1809-1816.
32. Watters JJ, Dorsa DM. Transcriptional effects of estrogen on neuronal neurotensin gene expression involve cAMP/protein kinase A-dependent signaling mechanisms. *J Neurosci*. 1998;18(17):6672-6680.
33. Morris NJ, Ross SA, Lane WS, et al. Sortilin is the major 110-kDa protein in GLUT4 vesicles from adipocytes. *J Biol Chem*. 1998;273(6):3582-3587.