N-terminal pro-Brain Natriuretic Peptide and Risk of Diabetes

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Brain natriuretic peptide (BNP) has an established role in cardiovascular disease. However, recent animal studies suggest direct metabolic effects of BNP. To determine the association of BNP with the risk of diabetes, we conducted a prospective analysis of participants from the Atherosclerosis Risk in Communities (ARIC) Study. We included 7,822 men and women without history of diabetes, cardiovascular disease or reduced kidney function at baseline. At baseline, N-terminal (NT)-proBNP, a cleavage product of BNP, was inversely associated with adiposity, fasting glucose, insulin, and cholesterol, but positively associated with blood pressure and C-reactive protein levels. During a median follow-up of 12 years, 1740 participants reported a new diagnosis of diabetes or medication use for diabetes. Baseline quartiles of NT-proBNP were inversely associated with diabetes risk, even after multivariable adjustment including fasting glucose. The adjusted HRs for diabetes were 1.0 (reference), 0.84 (95%CI 0.74- 0.96), 0.79 (95%CI 0.68- 0.90) and 0.75 (95%CI 0.64-0.87) for the 1st, 2nd, 3rd and 4th quartiles of baseline NT-proBNP, respectively (p-for-trend<0.001). This inverse association was robust across sex, race and obesity subgroups. Our results extend animal studies and support a direct and important metabolic role of BNP in humans.
Brain natriuretic peptide (BNP), one of the three natriuretic peptides released by the heart in response to hemodynamic stress, has an established role in cardiovascular (vasodilation) and renal (natriuresis) physiology, in which it confers protection against fluid overload and hypertension(1). It is widely accepted that BNP is closely associated with left ventricular mass and accurately detects heart failure(2). In the general population, elevated levels of BNP are associated with an increased risk of mortality and cardiovascular disease (CVD), especially heart failure(3; 4).

More recently, it has been postulated that the effects of natriuretic peptides extend beyond the cardiovascular system and that they may play a role in metabolic regulation, lipolysis, and the development of insulin resistance (5-7). Receptors for natriuretic peptides have been found in cells of tissues other than the classical cardiovascular and renal systems, including adipose tissue(8). In an experiment conducted by Miyashita et al., BNP transgenic mice and wild-type controls were exposed a high-fat and iso-caloric diet; compared to the control mice, the BNP transgenic mice exhibited less weight gain, less ectopic fat accumulation, and less insulin resistance. Further experiments shed some light into some potential mechanisms involved in the protection of these transgenic mice: increased oxygen utilization, increased mitochondrial content in skeletal muscle and increased gene expression of genes involved in fat oxidation and energy expenditure \( \text{[peroxisome proliferator-activated receptor (PPAR)-}\gamma\text{ coactivator (PGC)-1}\alpha \)\(}\)(6). Another animal study Bordicchia et al. extended these findings by demonstrating that treating mice with BNP show increased energy expenditure and increased thermogenic activation in brown and white adipose tissue(9). All together these studies suggest that BNP exert direct effects on mitochondrial and adipose tissue that can lead to increased glucose utilization and a decrease in adiposity.

Cross-sectional studies have shown low levels of BNP among persons who are overweight or obese (10-13) and, in some studies, lower BNP among adults with diabetes or the metabolic
syndrome (11; 12). To our knowledge the independent association between BNP levels and risk of diabetes has been addressed by two small studies in Europe and they found inconsistent results (14; 15).

The objective of this study was to determine the independent association between BNP levels and risk of diabetes in a community-based population without diabetes or cardiovascular disease at baseline. We hypothesized that elevated levels of N-terminal (NT)-proBNP, a stable cleavage product of BNP, would be associated with decreased risk of diabetes.

**METHODS**

**Study population**

The Atherosclerosis Risk in Communities (ARIC) Study is an ongoing community-based, predominantly bi-racial cohort of 15,792 middle-aged adults from 4 U.S. communities: Forsyth Country, North Carolina; Jackson, Mississippi; Minneapolis, Minnesota; and Washington County, Maryland (16). The first examination of participants took place from 1987 to 1989, with 3 follow-up visits taking place, each approximately 3 years apart, and a fifth visit currently taking place (2011-2013). The fourth visit (during 1996-1998) was attended by 11,656 and is the baseline for the present study. We excluded participants with race/ethnicity other than black or white (n=31), persons with a history of diabetes (n=1362), coronary heart disease or heart failure (n=1120), reduced kidney function (estimated glomerular filtration rate [eGFR] <60 mL/min/1.73 m$^2$) (n=623), and persons with missing data (n=555) or without an 8-hour fasting sample (n=143). The final sample size was 7,822 adults.

All participants signed written informed consent and the institutional review boards (IRB) at each clinical site approved the study.
Measurement of NT-proBNP

NT-proBNP was measured in stored at -70°C plasma samples collected from participants during visit 4 (1996-1998), using an electrochemiluminescent immunoassay on an automated Cobas e411 analyzer (Roche Diagnostics) with lower limit of detection ≤5 pg/mL and coefficient of variation 3.5–4.7%. Studies have demonstrated that NT-proBNP is a good and more stable marker of BNP, with long-term stability(17).

Assessment of Diabetes

Incident diabetes was defined as a self-reported physician diagnosis of diabetes or use of medications for diabetes identified during the annual telephone calls to all participants beginning after visit 4 (1996-1998) with follow-up through April 2011.

Other Measurements

Smoking history, alcohol consumption (never, former and current drinker, and usual ethanol intake [g/week] among current drinkers), medical history, medication use, and family history of diabetes were assessed during a home interview and according to a published protocol(18). We defined history of cardiovascular disease as self-reported myocardial infarction or stroke before visit 1, or silent myocardial infarction (diagnosed by electrocardiographic changes), adjudicated myocardial infarction or revascularization (at or before visit 4), and adjudicated hospitalization for congestive heart failure (at or before visit 4). Using standardized methods, height, weight, waist circumference, and blood pressure were measured. Fasting blood samples were obtained, and using standard methods the following assays were performed: serum glucose, insulin,
cholesterol levels (total, LDL- and HDL-cholesterol), triglycerides, creatinine, hs-C-reactive protein, plasma lactate, hematocrit(19). Cardiac troponin T (cTnT) was measured using a novel high-sensitivity assay, Elecsys Troponin T (Roche Diagnostics, Indianapolis, Indiana) with a lower limit of detection of 3.0 ng/l.

**Statistical Methods**

Baseline levels of NT-proBNP were categorized into quartiles. Baseline characteristics of the study population were summarized across quartiles of NT-proBNP. Participants with NT-proBNP below the limit of detection (n=248 [3.2%]) were assigned a value of 2.5 pg/mL. We used the Kaplan-Meier method to show the differences in the overall risk (cumulative incidence) of diabetes by baseline quartiles of NT-proBNP. Adjusted hazard ratios and their confidence intervals were estimated using Cox proportional hazards model. We implemented four core models. Model 1 included age, race/center, sex and education. Model 2 included all variables in Model 1 plus body mass index (linear spline with a knot at 30 kg/m$^2$). Model 3 included all variables in Model 2 plus height (in meters) squared, systolic and diastolic blood pressure, and high sensitivity C-reactive protein. Model 4 included all variables in Model 3 plus fasting glucose and family history of diabetes. We tested for interactions with race, sex and obesity status.

We conducted sensitivity analyses excluding: 1) persons with elevated high sensitivity-cTnT (defined as levels above the 99th percentile value [14 ng/l] in a healthy subpopulation ages 20 to 70 years(19)) ; 2) participants with impaired fasting glucose or undiagnosed diabetes (fasting glucose ≥100 mg/dl) at baseline. We also conducted competing risk regression analyses to examine the possible effect of competing mortality on our results(20).
To characterize the shape of the association of NT-proBNP with incident diabetes in our adjusted Cox regression model, we implemented a piece-wise linear spline model, with knots at the 33\textsuperscript{rd} and 66\textsuperscript{th} percentiles. In this model, we truncated the data at percentile 99\textsuperscript{th}, and the hazard ratio at the 10 percentile of NT-proBNP was used as the reference.

**RESULTS**

Baseline characteristics of the study population are shown in **Table 1**. Participants in the lowest quartile of NT-proBNP at baseline were younger, less likely to be white, female, current smokers and were more likely to have an adverse lipid profile, higher body mass index and waist circumference, and higher levels of fasting glucose, insulin and lactate compared to participants in the highest quartile of NT-proBNP. In contrast, participants in the lowest quartile of NT-proBNP had lower levels of high sensitivity C-reactive protein, systolic blood pressure and lower prevalence of hypertension and elevated high sensitivity cardiac troponin T compared to participants in the upper quartile.

During a median follow-up for 12 years, there were 1740 new cases of self-reported diabetes among the 7822 persons in the study population at baseline. The cumulative incidence of diabetes by quartile of baseline NT-proBNP is shown in **Figure 1**. The unadjusted incidence rates are shown in the Online Appendix (**Supplemental Table 1**).

Persons in the lowest quartile of NT-proBNP had a significantly higher risk of diabetes compared to persons in the upper quartiles, even after multivariable adjustment (**Table 2**). In Model 1, the adjusted HRs of diabetes were 1.0 (reference), 0.88 (95\%CI 0.77-0.99), 0.76 (95\%CI 0.66-0.87) and, 0.67 (95\%CI 0.58-0.78), for 1\textsuperscript{st}, 2\textsuperscript{nd}, 3\textsuperscript{rd} and 4\textsuperscript{th} quartile respectively (P value for trend<0.001). After additional adjustment for body mass index and cardiovascular risk...
factors the associations were attenuated, but remained statistically significant. The risk of diabetes remained significantly lower in all three upper quartiles compared to the first quartile of NT-proBNP even after further adjustment for baseline fasting glucose (Model 4).

Consistent with the results from the models of NT-proBNP in quartiles, our spline model showed a threshold effect with the steepest and significant decline in diabetes risk at the NT-proBNP levels 14.7-24.9 pg/mL (p value =0.02), with a shallower and non significant slope at higher levels of NT-proBNP. Indeed the slope was strongly significant (p value >0.05) (Figure 2).

We did not observe any significant interactions by sex, race or obesity (Table 3).

In sensitivity analyses, excluding 438 ARIC participants with undiagnosed diabetes or impaired fasting glucose at baseline, the results were very similar or even strengthened, with HRs for diabetes of 1.0 (reference), 0.88 (95%CI 0.76-1.02), 0.80 (95%CI 0.69-0.94) and 0.71 (95%CI 0.60-0.84) for those in the 1\textsuperscript{st}, 2\textsuperscript{nd}, 3\textsuperscript{rd} and 4\textsuperscript{th} quartile, respectively. Similarly, after exclusion of 386 participants with elevated high sensitivity cardiac Troponin T, the results were not appreciably altered (Supplemental Table 2). Finally, using competing risk models the inverse association of NT-proBNP with diabetes was strengthened. After accounting for competing mortality in Model 4, the HRs (95% CIs) for diabetes in the 1\textsuperscript{st}, 2\textsuperscript{nd}, 3\textsuperscript{rd} and 4\textsuperscript{th} quartile were 1.0 (reference), 0.81 (0.71-0.93), 0.67 (0.57-0.79) and 0.61 (0.52-0.72) respectively.

**DISCUSSION**

Our findings show that in a community-based population without diabetes, cardiovascular disease (CHD or CHF) or reduced renal function at baseline, higher levels NT-proBNP were associated with a significantly decreased risk of diabetes, even after adjustment for traditional risk factors and fasting glucose. Our results were consistent across race, sex, and body mass
index categories. Furthermore, the association remained significant after excluding people with impaired fasting glucose at baseline. These results are consistent with limited animal and in-vivo studies that suggest circulating levels of BNP levels are metabolically active.

The mechanisms by which BNP and related peptides may influence adipose and glucose metabolism are not well understood. Studies conducted so far have demonstrated direct effects of BNP on muscle and adipose tissue that could at least, in part, influence the development of insulin resistance. Miyashita et al., showed that BNP transgenic mice with overexpression of BNP, had decreased risk of obesity and insulin resistance after receiving a high-fat diet, and increased muscle mitochondrial content and fat oxidation. The authors suggest that the cellular effects of BNP emulate the effects observed through catecholamines and include: binding to the natriuretic peptide receptor A (NPR-A) in adipocytes, activation of the cGMP cascade, downstream activation of cGMP-dependent protein kinase (PKG) and p38MAPK which results in increased mitochondrial biogenesis and uncoupled respiration(6). More recently, Bordicchia et al demonstrated in a series of animal and in vivo experiments that natriuretic peptides (BNP and ANP) activate “brown adipose tissue-like” mechanisms involved with thermogenesis but in white adipose tissue(9; 21). In an experimental study in humans, Heinisch et al. administered intravenously BNP or placebo to 20 healthy volunteers and performed an intra venous glucose tolerance test. Their results showed that after infusion of BNP there were reduced levels of circulating glucose, but no effect on insulin secretion and sensitivity(5).

To our knowledge little work has been done examining the inter-relationship between cardiovascular and noncardiovascular effects of BNP as they pertain to the etiology or development of insulin resistance. In our study, participants in the lowest quartile of NT-proBNP had higher levels of lactate compared to participants in the highest quartile of NT-proBNP suggesting that blood flow and oxygen delivery, may play into the development of insulin resistance. Future studies should be conducted to test this hypothesis.
Most previous epidemiological studies of NT-proBNP and diabetes were cross-sectional and focused on potential mechanisms for the “natriuretic handicap” which refers to the reduced levels of BNP seen in people with obesity, which in turn, could lead to an increased susceptibility for hypertension and heart failure (10; 22). It has been postulated that there is increased clearance of BNP by the NPR-C receptor in adipose tissue. However, recent studies have shown that NT-proBNP is cleared by kidney elimination and not by binding to the clearance receptor (NPR-C) (8). Nonetheless, our results were consistent after excluding persons with reduced kidney function and among persons with normal body mass index.

To our knowledge data examining the prospective association between natriuretic peptides and diabetes risk in humans are limited. In Sweden, using data from the Malmo Diet and Cancer study, investigators examined the association between atrium natriuretic peptide (ANP) and NT-proBNP and incident diabetes in a sample of 1828 adults (301 cases of diabetes). The authors found a significant and inverse association between ANP and the risk of diabetes, and an inverse but not statistically significant association between NT-proBNP and diabetes risk (OR=0.95, 95% CI 0.84-1.08 comparing the 4th quartile of NT-proBNP to the 1st quartile)(14). In a study using data from the FINRISK97 study (n=7827) with 417 cases of incident diabetes, there was a significant inverse association between BNP and diabetes(23). A case-cohort study with 440 cases and 740 controls drawn from the EPIC-Norfolk Study, there was a significant inverse association between NT-proBNP and diabetes risk (HR of 0.82; 95% CI 0.69-0.97 per 1 SD increase in NT-proBNP)(15). Finally, Pfister et al., combined the results of the EPIC-Norfolk Study with small case-control studies with genotype information and demonstrated that a common genetic variant (rs198389) within the genome region that encodes BNP was associated with a reduced risk of type 2 diabetes(15).
Our study has some limitations that should be considered in the interpretation of these results. We only had information on new cases of diabetes defined by self-reported diagnosis or diabetes medication use, which is a highly specific definition of diabetes but has relatively low sensitivity (24). We also had only single measurements of NT-pro-BNP at baseline and it has been found that all natriuretic peptides have relatively high intra-individual variation (25). This introduces random error to the classification of participants and most likely biased the results toward the null. As with any observational study, the possibility of residual confounding cannot be completely eliminated. Although we had information on fasting glucose, and the results were consistent after adjusting for it; we lacked information on oral glucose tolerance test and therefore it is no possible to test whether NT-pro-BNP predicts diabetes independently of two-hour oral glucose tolerance test. Our study also has a number of important strengths including the prospective design, long term follow-up (median of 12 years), large bi-racial community-based sample with a large number of cases of diabetes occurring during the follow-up (N=1740), and the rigorous measurements of known risk factors for diabetes using standardized protocols.

In conclusion, our results suggest that persons with very low levels of BNP have a significantly higher risk of developing diabetes compared to persons with moderately high levels of NT-proBNP in a general population. These results provide additional evidence of a novel role for natriuretic peptides in metabolic disorders. Given the availability of recombinant human BNP (26; 27), more studies are needed to determine their usefulness at preventing the development of diabetes.

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"Dr. Mariana Lazo is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis."

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Table 1. Characteristics of study population* according to quartiles of NT-proBNP at baseline

| NT-proBNP quartile [range, pg/mL] | Q1 [<31] N=1953 | Q2 [31.1-61.8] N=1952 | Q3 [61.9-114.4] N=1957 | Q4 [>114.4] N=1960 | P value for linear trend† |
|-----------------------------------|-----------------|------------------------|------------------------|-----------------|------------------------|
| n                                 |                |                        |                        |                 |                        |
| Age, years                        | 60.4 (5.0)     | 61.6 (5.3)             | 62.7 (5.4)             | 64.0 (5.7)      | <0.001                 |
| Female, %                         | 38.8           | 56.2                   | 67.8                   | 73.5            | <0.001                 |
| White, %                          | 67.5           | 79.8                   | 83.8                   | 87.2            | <0.001                 |
| Current smoker, %                 | 13.8           | 15.4                   | 13.0                   | 16.3            | 0.006                  |
| LDL-cholesterol, mg/dl            | 128.5 (34.4)   | 125.9 (32.8)           | 121.6 (31.3)           | 118.2 (32.3)    | <0.001                 |
| HDL-cholesterol, mg/dl            | 47.5 (14.6)    | 50.5 (15.9)            | 53.9 (17.0)            | 55.4 (17.7)     | <0.001                 |
| Triglycerides >150mg/dl, %        | 32.7           | 32.6                   | 27.7                   | 29.4            | 0.001                  |
| BMI, kg/m²                        | 29.3 (5.0)     | 28.6 (5.5)             | 28.1 (5.5)             | 27.6 (5.7)      | <0.001                 |
| BMI >30 kg/m², %                  | 37.6           | 32.3                   | 29.7                   | 27.4            | <0.001                 |
| BMI ≤20 kg/m², %                  | 0.6            | 1.7                    | 2.8                    | 4.6             | <0.001                 |
| Weight, kg                        | 177.8 (31.3)   | 169.8 (35.7)           | 163.6 (33.1)           | 160.6 (35.3)    | <0.001                 |
| Height, m                         | 1.70 (0.09)    | 1.68 (0.09)            | 1.66 (0.09)            | 1.65 (0.08)     | <0.001                 |
| Waist, cm                         | 96.7 (11.4)    | 95.0 (13.0)            | 93.5 (13.3)            | 92.8 (13.9)     | <0.001                 |
| High Waist Circumference‡         | 62.7           | 64.5                   | 63.5                   | 61.8            | 0.42                   |
| Waist to hip ratio                | 0.96 (0.06)    | 0.94 (0.07)            | 0.93 (0.08)            | 0.92 (0.08)     | <0.001                 |
| Fasting Glucose, mg/dL            | 101 [95-109]   | 99 [94-107]            | 97 [92-104]            | 96 [90-103]     | <0.001                 |
| Family History Diabetes          | 22.1           | 23.8                   | 21.9                   | 19.7            | 0.02                   |
|                              | 50.5 | 39.8 | 35.3 | 31.7 | <0.001 |
|------------------------------|------|------|------|------|--------|
| **Impaired Fasting Glucose, % §** |      |      |      |      |        |
| **Insulin**                  |      |      |      |      | <0.001 |
| Median                       | 11.8 | 10.1 | 9.1  | 8.6  |        |
| IQR                          | [8.4-16.6] | [7.1-14.5] | [6.4-13] | [5.8-12.1] |  |
| **Lactate, mg/dL**           |      |      |      |      | <0.001 |
| Median                       | 6.8  | 6.4  | 6.3  | 6.1  |        |
| IQR                          | [5.5-8.7] | [5.2-8.3] | [5.1-7.9] | [5-7.8] |  |
| **C-reactive protein, mg/L** |      |      |      |      | <0.001 |
| Median                       | 2.0  | 2.1  | 2.2  | 2.6  |        |
| IQR                          | [0.95-4.42] | [0.98-4.77] | [1.0-5.0] | [1.1-5.8] |  |
| **Hypertension,%**           | 24.6 | 22.5 | 26.6 | 32.7 | <0.001 |
| **SBP, mmHg**                | 122.3 (15.3) | 123.9 (16.8) | 126.9 (18.9) | 131.3 (20.6) | <0.001 |
| **DBP, mmHg**                | 72.4 (9.4) | 71.3 (9.4) | 70.8 (10.1) | 71.0 (10.9) | <0.001 |
| **Elevated hs-cTnT, %**      | 4.1  | 3.8  | 5.1  | 6.8  | <0.001 |
| **Hematocrit, % ||**         | 41.7 (3.3) | 40.6 (3.2) | 39.6 (3.3) | 38.8 (3.4) | <0.001 |

Data presented as mean(SD) unless indicated. IQR= Interquartile range.

*N=7822 participants of the ARIC Study without a history of diabetes, coronary heart disease, congestive heart failure or kidney disease. † Linear test using quartiles numbers. ‡ Waist circumference > 102 cm or 88 for men and women, respectively. § Fasting plasma glucose 100-125 mg/dL. || Among non current smokers.
Table 2. Adjusted Hazard Ratios (95% Confidence Intervals) of Incident Diagnosed Diabetes by Quartiles of NT-proBNP at Baseline

| NT-proBNP quartile [range, pg/mL] | Q1 [<31] N=1953 | Q2 [31.1-61.8] N=1952 | Q3 [61.9-114.4] N=1957 | Q4 [>114.4] N=1960 | P value for linear trend* |
|-----------------------------------|------------------|-----------------------|------------------------|---------------------|-------------------------|
| Model 1 † (Reference)             | 1.0              | 0.88                  | 0.76                   | 0.67                | <0.001                  |
| Model 2 ‡ (Reference)             | 1.0              | 0.92                  | 0.83                   | 0.76                | <0.001                  |
| Model 3 § (Reference)             | 1.0              | 0.88                  | 0.76                   | 0.65                | <0.001                  |
| Model 4 ¶ (Reference)             | 1.0              | 0.84                  | 0.79                   | 0.75                | <0.001                  |

* Linear test using quartiles numbers
† Model 1: Age, sex, race/ARIC center, education
‡ Model 2: Model 1+ BMI (linear spline with a knot at 30 kg/m²)
§ Model 3: Model 2+ height²+ SBP, DBP, smoking, LDL, HDL, hs-CRP
¶ Model 4: Model 3+ fasting glucose and family history of diabetes
Table 3. Adjusted* Hazard Ratios (95% Confidence Intervals) of Incident Diagnosed Diabetes by Quartiles of NT-proBNP at Baseline in Population Subgroups

| NT-proBNP quartile [range, pg/mL] | Q1 [<31] | Q2 [31.1-61.8] | Q3 [61.9-114.4] | Q4 [>114.4] | P value for linear trend† | P-for-Interaction |
|-----------------------------------|----------|----------------|----------------|------------|--------------------------|------------------|
| Race                              |          |                |                |            |                          |                  |
| Black                             | 1.0      | 0.89           | 0.80           | 0.71       | 0.02                     | 0.87             |
| N=1598 Reference                  |          | (0.71-1.12)    | (0.61-1.03)    | (0.52-0.97)† |                          |                  |
| White                             | 1.0      | 0.81           | 0.78           | 0.75       | 0.002                    |                  |
| N=6224 Reference                  |          | (0.69-0.95)†  | (0.66-0.92)†  | (0.62-0.89)† |                          |                  |
| Sex                               |          |                |                |            |                          |                  |
| Men                               | 1.0      | 0.82           | 0.87           | 0.82       | 0.11                     | 0.55             |
| n=3201 Reference                  |          | (0.68-0.99)†  | (0.71-1.09)    | (0.63-1.06) |                          |                  |
| Women                             | 1.0      | 0.84           | 0.72           | 0.69       | <0.001                   |                  |
| N=4621 Reference                  |          | (0.71-1.01)    | (0.60-0.87)†  | (0.56-0.84)† |                          |                  |
| Age                               |          |                |                |            |                          |                  |
| <62 years                         | 1.0      | 0.82           | 0.82           | 0.63       | <0.001                   | 0.28             |
| N=3846 Reference                  |          | (0.69-0.98)†  | (0.67-0.99)†  | (0.49-0.79)† |                          |                  |
| ≥ 62 years                        | 1.0      | 0.83           | 0.74           | 0.82       | 0.05                     |                  |
| N=3976 Reference                  |          | (0.68-1.00)†  | (0.60-0.91)†  | (0.66-1.01)|                          |                  |
| Obesity                           |          |                |                |            |                          |                  |
| <30 kg/m²                         | 1.0      | 0.94           | 0.82           | 0.80       | 0.02                     | 0.52             |
| N=5340 Reference                  |          | (0.79-1.14)    | (0.67-1.00)†  | (0.64-0.99)† |                          |                  |
| ≥30 kg/m²                         | 1.0      | 0.76           | 0.76           | 0.69       | 0.02                     |                  |
| N=2482 Reference                  |          | (0.63-0.91)†  | (0.63-0.92)†  | (0.56-0.86)† |                          |                  |
* Adjusted for: Age, sex, race, ARIC center, education, BMI, height², SBP, DBP, smoking, LDL, HDL, hs-CRP, fasting glucose and family history of diabetes

† Linear test using quartiles numbers

‡ p<0.05
Figure 1. Cumulative Incidence (%) of Diagnosed Diabetes by Quartiles of NT-proBNP at baseline
Figure 2. Adjusted* hazard ratio (95% confidence interval) of incident diagnosed diabetes by baseline NT-ProBNP level overlaid on the distribution (frequency histogram) of NT-ProBNP in the study population

Hazard ratios are presented using a logarithmic scale. Linear spline centered at 14.7 (percentile 10th), with knots at 33 and 66th percentiles (24.9 and 76.2, respectively), after excluding values ≥99 percentile (NT-proBNP ≥611, pg/mL).

Adjusted for weight, height, age, race/center, sex, systolic blood pressure, diastolic blood pressure, smoking, LDL cholesterol, HDL cholesterol, C-reactive protein, fasting glucose and family history of diabetes.
Supplemental Table 1. Incidence rate of diagnosed diabetes by quartiles of NT-proBNP at baseline

| NT-proBNP quartile [Range, pg/mL] | Q1 [<31] N=1953 | Q2 [31.1-61.8] N=1952 | Q3 [61.9-114.4] N=1957 | Q4 [>114.5] N=1960 |
|-----------------------------------|----------------|------------------------|------------------------|---------------------|
| Person-years of follow up         | 19489.19       | 19804.26               | 19956.01               | 19848.89            |
| Cases of Diagnosed Diabetes      | 539            | 461                    | 397                    | 343                 |
| Incidence rate (per 1000 person-years) | 27.66 | 23.28                  | 19.89                  | 17.28               |
Supplemental Table 2. Adjusted$^a$ Hazard Ratios (95% Confidence Intervals) of Incident Diagnosed Diabetes by Quartile of NT-proBNP at Baseline After Additional Exclusions

| NT-proBNP quartile [Range, pg/mL] | Q1 [<31] | Q2 [31.1-61.8] | Q3 [61.9-114.4] | Q4 [>114.5] |
|-----------------------------------|---------|----------------|----------------|-------------|
| Excluding people with elevated hs-cTnt N=386 | 1.0 Reference | 0.84 (0.73-0.96) | 0.78 (0.68-0.90) | 0.71 (0.61-0.83) |
| Excluding people with undiagnosed diabetes N=438 | 1.0 Reference | 0.88 (0.76-1.02) | 0.80 (0.69-0.94) | 0.71 (0.60-0.84) |

$^a$ Adjusted for: Age, sex, race, ARIC center, education, BMI, height$^2$, SBP, DBP, smoking, LDL, HDL, hs-CRP, fasting glucose and family history of diabetes