Elevated Plasma Levels of Nt-proBNP in Patients With Type 2 Diabetes Without Overt Cardiovascular Disease

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OBJECTIVE — The NH_2-terminal portion of the precursor of brain natriuretic peptide (Nt-proBNP) has been reported to be elevated in left ventricular dysfunction. This peptide is a split product from the proBNP molecule, and its level in the circulation is not, as the mature BNP peptide, dependent on the peripheral number of BNP receptors. We aimed to test the hypothesis that asymptomatic left ventricular dysfunction (ALVD), as estimated by Nt-proBNP, would be more prevalent in patients with type 2 diabetes without overt cardiovascular disease in comparison with matched control subjects.

RESEARCH DESIGN AND METHODS — The study population consisted of 253 patients with type 2 diabetes and 230 matched control subjects aged 40–70 years without any overt heart disease from primary care centers in Western Finland and Southern Sweden. Nt-proBNP was measured in plasma by competitive enzyme immunosorbent assay.

RESULTS — Patients with type 2 diabetes were shown to have higher Nt-proBNP values (360.9 pmol/l [262.6–467.9]) than control subjects (302.7 pmol/l [215.4–419.2]) ($P < 0.001$). Nt-proBNP levels were independently related to diabetes after adjustment for age, sex, systolic and diastolic blood pressure, BMI, heart rate, drug treatment, serum creatinine, and cystatin C.

CONCLUSIONS — Our data suggest that the secretion of Nt-proBNP is increased in type 2 diabetic patients with no overt heart disease, suggesting that type 2 diabetes is associated with a higher prevalence of ALVD than hitherto thought. Nt-proBNP may thus serve as a screening instrument to select patients with type 2 diabetes who could benefit from an echocardiographical examination.

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The incidence and prevalence of type 2 diabetes increases worldwide. In the adult population all over the world, the average prevalence for diabetes is estimated to be at least 4.0% (1). This figure is predicted to double until the year 2015 (2). Although microangiopathy represents a severe threat to the population with diabetes, macroangiopathy and subsequent cardiovascular disease are the major causes of morbidity and mortality in these patients. Screening for kidney and retinal complications is already an established part of routine diabetes care today, but there is no comparable reoccurring screening for cardiac complications of diabetes. This may simply be due to the lack of cost-effective methods; an echocardiographical examination is both expensive and time consuming and, therefore, not suited for screening purposes. The most evident cardiac complication is coronary atherosclerosis. Not only is the extent of coronary atherosclerosis increased, the disease becomes clinical earlier and is more generalized in the coronary tree compared with the subjects without diabetes (3). Diabetes is also more prevalent among patients with heart failure. In the Framingham study, male patients with diabetes had twice the risk and female patients five times the risk of a control population to develop heart failure (4). At least partially, this could be explained by the increase in severity and incidence of ischemic heart disease among patients with diabetes. However, data from autopsy studies have suggested that hearts from patients with diabetes also have an increased collagen content (5). Moreover, patients with diabetes have a disproportional increase in left ventricular mass independent of blood pressure (6–8). All of these factors may contribute to increased myocardial stiffness. This is especially important because left ventricular hypertrophy in a meta-analysis has been associated with a 1.5- to 3.5-fold increased risk of future cardiovascular morbidity and a 1.5- to 6.8-fold increase of all-cause mortality (9). Thus, taken together, there are several mechanisms beside the more aggressive atherosclerosis that could explain why patients with diabetes have a higher cardiac morbidity and mortality.

Brain natriuretic peptide (BNP) is a 32–amino acid peptide (10). It is synthesized predominantly in the left ventricle of the heart as the 108–amino acid prohormone preproBNP (γ-BNP) (11–13). The hormone is a potent vasodilator and a natriuretic factor regulating salt and water homeostasis. BNP is stored in the human cardiac tissue mainly as BNP-32 with a lesser amount of the precursor pre-

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Abbreviations: ALVD, asymptomatic left ventricular dysfunction; BNP, brain natriuretic peptide; LVD, left ventricular dysfunction; Nt-proBNP, NH_2-terminal portion of the precursor of BNP; SBP, systolic blood pressure.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Demographic data for the study population*

	Patients with type 2 diabetes	Control subjects	*P*
n	253	230	
Age (years)	59.6 (54.3–63.9)	58.1 (51.7–63.4)	0.256
Sex (% women)	51.4	60.4	0.072
BMI (kg/m^2)	28.3 (25.5–32.4)	25.8 (24.1–28.1)	<0.001
SBP (mmHg)	140.0 (130.0–158.0)	130.0 (120.0–144.0)	<0.001
Diastolic blood pressure (mmHg)	84.0 (78.0–90.0)	80.0 (74.0–90.0)	<0.003
Pulse (s^{-1})	70 (64–78)	66 (60–72)	<0.001
Creatinine (μmol/l)	84.0 (76.0–95.3)	86.0 (77.0–95.3)	0.352
Cystatin C (g/l)	1.05 (0.980–1.17)	1.09 (1.00–1.24)	0.034
Cholesterol (mmol/l)	5.61 (5.03–6.48)	5.84 (5.21–6.73)	0.061
LDL cholesterol (mmol/l)	3.56 (2.95–4.26)	3.86 (3.22–4.56)	0.007
HDL cholesterol (mmol/l)	1.18 (0.99–1.41)	1.35 (1.16–1.67)	<0.001
Triglycerides (mmol/l)	1.70 (1.20–2.32)	1.20 (0.90–1.63)	<0.001

Data are means (SD range).

proBNP. The circulating plasma forms of BNP are BNP-32 and the NH_2-terminal portion proBNP (Nt-proBNP) (1-76) (14,15). Increased secretion of BNP and Nt-proBNP occurs mainly with increased tension in the ventricular walls, decreased oxygen supply, acute myocardial infarction, chronic cardiac heart failure, and in hypertrophy of the heart (16,17).

In a head-to-head comparison study by Hammerer-Lercher et al. (18), BNP and Nt-proBNP were found to be superior markers to Nt-pro atrial natriuretic peptide in detecting left ventricular dysfunction (LVD).

Cardiovascular death accounts for ~70% of the deaths among subjects with diabetes. The treatment of LVD, even when asymptomatic, is associated with a better prognostic outcome (19). This study was performed to establish whether asymptomatic LVD (ALVD), as estimated by Nt-proBNP, is overrepresented in patients with type 2 diabetes compared with nondiabetic control subjects for both groups without overt cardiovascular disease.

RESEARCH DESIGN AND METHODS

— We compared plasma concentrations of Nt-proBNP between 253 subjects with and 230 without type 2 diabetes. None of the subjects had any known cardiovascular disease. The patients were recruited from health care centers in the Botnia region in Western Finland, Southern Finland, and Southern Sweden (20). Type 2 diabetes was diagnosed according to the World Health Organization definition from 1985 (21). Kidney function was normal, and none of the patients had any nephropathy either by report or by creatinine/cystatin C values. No subjects were under drug treatment with digitalis or nitrates. The characteristics of the study population are presented in Table 1.

Patient recruitment and inclusion visit was performed as described earlier for the Botnia study. Briefly, all patients underwent medical examination by a physician. A careful medical history was taken to obtain information about other diseases (particularly hypertension, coronary heart disease, myocardial infarction, stroke, peripheral vascular disease, and endocrine disorders) and medication. Body weight and height were measured with subjects in light clothing without shoes. Three blood pressure recordings were obtained from the right arm while in the supine position after 30 min of rest at 5-min intervals, and their mean value was calculated. Blood samples were drawn into Vacutainer tubes containing EDTA. Plasma was frozen at −80°C for the measurement creatinine, cystatin C, and Nt-proBNP.

Nt-proBNP assay

Nt-proBNP was analyzed using a competitive enzyme immunosorbent assay designed to measure the immunoreactive Nt-proBNP (Biomedica Laboratories, Vienna, Austria). The cutoff value for LVD was set to 350 pmol/l, according to an earlier study by Hughes et al. (22). This is in accordance to most of the levels cited in the literature.

Cystatin C assay

Plasma cystatin C was measured by a fully automated particle-enhanced turbidimetric assay on undiluted samples (23,24) using a Behring BN ProSpec analyzer (Dade Behring, Deerfield, IL) and a calibrator (24) obtained from DakoCytomation (Glostrup, Denmark).

Statistics

Analyses were performed in statistical software package SigmaStat 2.0. Demographic data were initially described as the median value of groups and the 25th and 75th percentiles. Significance of differences between groups was tested by a Mann-Whitney rank-sum test with $P < 0.05$ considered as statistically significant. Due to the skewed distribution of Nt-proBNP values, ln(Nt-proBNP) was used in a multiple logistic regression model with diabetic phenotype as outcome. A χ^2 analysis was performed to test significance of frequency differences.

RESULTS

— Patients with diabetes had a higher median value of Nt-proBNP (360.9 pmol/l [262.6–467.9]) than the population without diabetes (302.7 pmol/l [215.4–419.2]) ($P < 0.001$) (Fig. 1). The proportion of individuals with a Nt-proBNP value above the cutoff value (350 pmol/l) was significantly higher (61.3 vs. 45.1%) among the diabetic patients than in the control group, according to a χ^2 analysis ($P < 0.001$).

First, a multiple logistic regression analysis model taking sex, age, pulse, BMI, cystatin C, and systolic and diastolic blood pressure into account was used. Then, the analysis was reperformed, taking only age and sex into account.

The odds ratio (OR) for diabetes and 1 SD change in parameters was calculated. Systolic blood pressure (SBP), pulse, BMI, cystatin C, and ln(BNP) were all independently influencing the risk of diabetic phenotype for 1 SD of change in parameters. For ln(Nt-proBNP), the OR was 1.60 (95% CI 1.26–2.03). This OR was only mildly changed when the multiple regression analysis was reperformed, taking only sex and age into account 1.54 (1.25–1.90) (Table 2).

Nt-proBNP values showed no correlation to HbA_{1c}, age, or SBP in the patients with diabetes (data not shown). However,

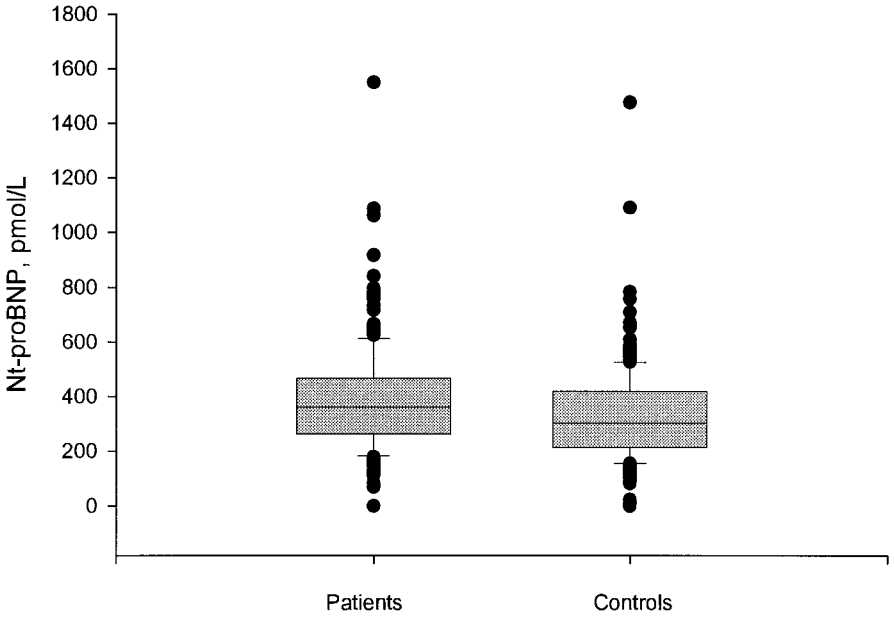

Figure 1—*Distribution of Nt-proBNP values in the patient* (n = *253) and control* (n = *230) cohorts.* P < *0.001 for difference between groups.*

high blood pressure has been implicated as being a confounder to Nt-proBNP values. Therefore, we reperformed analyses of Nt-proBNP values between individuals above and below an SBP of 135 mmHg. There was no statistical difference between the two groups in the control population ($P = 0.483$). There was, however, a statistical difference within the diabetes group, such that the patients with SBP <135 mmHg had higher Nt-proBNP values (401.0 [290.5–504.9]) than their hypertensive (SBP >135 mmHg) peers ($P < 0.027$).

The trend of change for Nt-proBNP to age was estimated in a linear regression with Nt-proBNP as a dependent variable and age as independent. Relation of HbA_{1c}, BMI, and SBP were for each factor individually analyzed by a linear regression in the patient group, with Nt-proBNP as the dependent variable and each of the factors used as independent variables.

Nt-proBNP change over age in the control population followed a regression curve Nt-proBNP = 90.099 + (4.044 × age). Thus, for each year of increase in age, the Nt-proBNP-level increased 4.04 pmol/l. Interestingly enough, no such trend was seen in patients with diabetes.

No differences in Nt-proBNP levels were detected between individuals on β-blockers, diuretics, and angiotensinogen inhibitors compared with untreated subjects. However, individuals on Ca-blocker treatment had significantly higher Nt-proBNP values (417.4 [277.8–530.1]) versus nontreated subjects (333.7 [229.3–444.8]) ($P = 0.015$). However, if Mann-Whitney's rank-sum test was reperformed on Nt-proBNP values between the control subjects and patients with diabetes with the omission of subjects on Ca-blockers, the differences between groups were consistent and still highly significant ($P < 0.001$).

Table 2—*OR for diabetes outcome*

Parameters	OR for 1 SD (95% CI)
SBP	1.86 (1.40–2.49)
DBP	0.791 (0.600–1.05)
Pulse	1.53 (1.21–1.93)
BMI	1.86 (1.44–2.39)
Cystatin C	0.809 (0.644–1.02)
ln(BNP)	1.60 (1.26–2.03)
ln(BNP)*	1.54 (1.25–1.90)

Data are OR (95% CI) for 1 SD change in a multiple regression model with SBP, diastolic blood pressure (DBP), pulse, BMI, cystatin C, ln(BNP), age, and sex taken into account. *Representing estimation of OR for 1 SD change only taking age and sex into account.

CONCLUSIONS — BNP has been shown to be elevated in early left ventricular systolic as well as in diastolic dysfunction (25). Nt-proBNP is a split product from the BNP. It is more stable, and the circulating concentration is not dependent on the receptor population in the individual patient. An increase in BNP might also be dependent on a downregulation of the receptor population, as has been suggested for patients with nephropathy (26). This should not be the case for Nt-proBNP, which is solely eliminated through glomerular filtration.

In our study, the Nt-proBNP level was shown to be significantly elevated in the cohort of patients with diabetes. However, patients with diabetes have a higher BMI, heart rate, and systolic and diastolic blood pressure than the control subjects, which might confound our results. In a multiple logistic regression model taking diabetes status as an outcome, ln(Nt-proBNP) was identified as an independent variable, even when the previously described possible confounders for BNP levels, systolic and diastolic blood pressure, sex, age, pulse, BMI, and cystatin C were taken into account. In addition, the OR was only mildly changed (and even lower) if the multiple regression analysis was reperformed with only sex and age taken into account.

There was in our material a considerable overlap between the two groups. This might partially be due to ALVD among the control population. In the Rotterdam study, systolic dysfunction was reported in 4% of the population aged 55–95 years. However, 60% of these were asymptomatic (27). Diastolic abnormality, as defined by the European Study Group on Diastolic Heart Failure, is much more prevalent, 11.1%, according to a substudy of the MONICA project. However, it differed between different age-groups, with 2.8% in those aged 25–35 years and 15.8% in subjects >65 years of age (28). In previous studies, BNP has been shown to be an early determinant of both diastolic and systolic dysfunction. In animal models, BNP gene expression has been shown to reflect ventricular and atrial pressures (29). In that study, even the animals with a mild compensated heart failure had elevated BNP levels. Thus, the Nt-ProBNP measurement, in addition to being an estimation of the combined diastolic and systolic performance, might also be a more sensitive measurement of ventricular disturbances than the echocardiographical examination. This might explain why, in our ma-

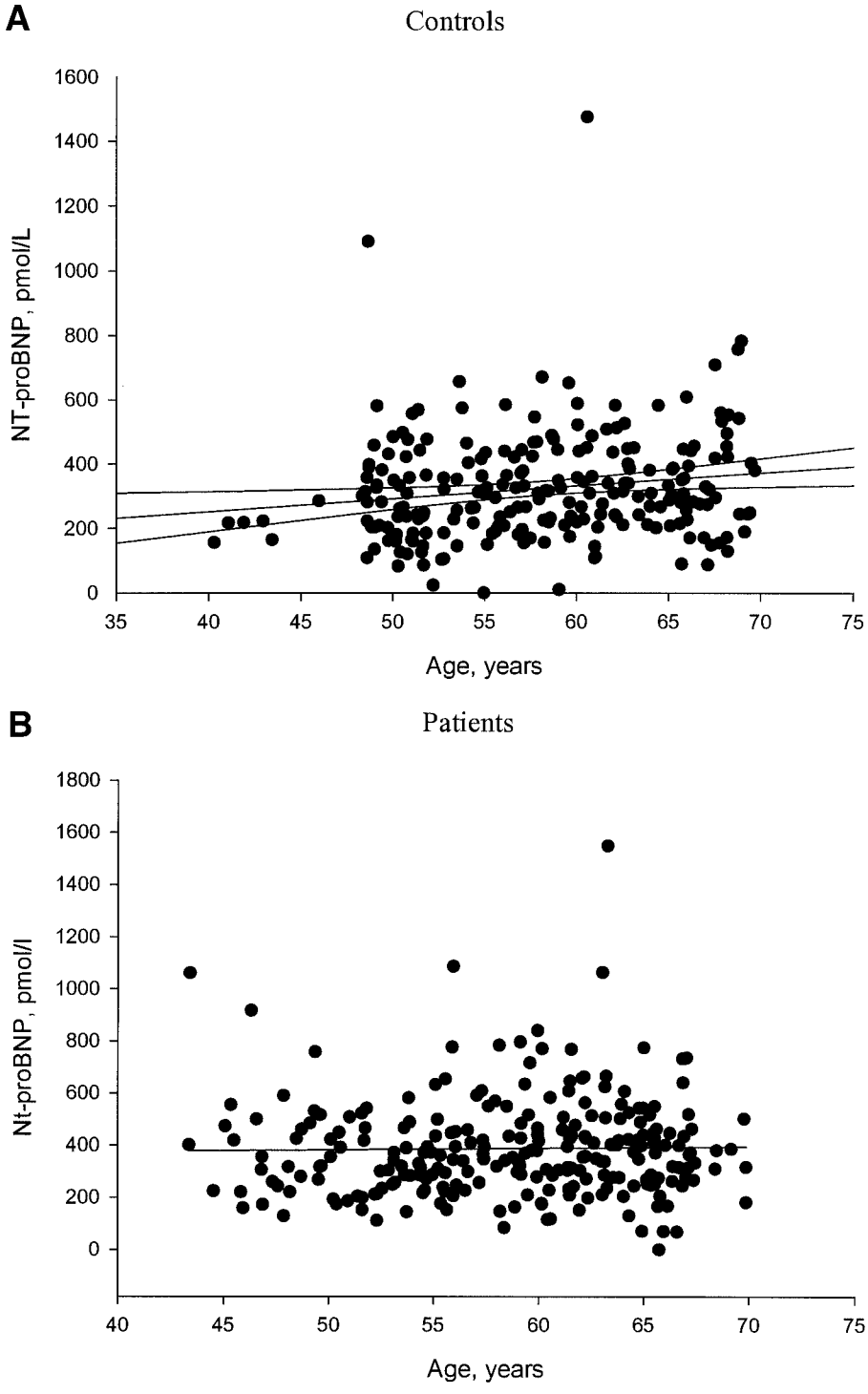

Figure 2—*Levels of Nt-proBNP in relation to age in the control* (A) *and the patient* (B) *cohorts. The linear regression curve for the nondiabetic subjects is Nt-proBNP = 90.099 + (4.044 × age).*

terial, 45% of the nondiabetic subjects had elevated levels of Nt-proBNP. Despite this suggested sensitivity for ventricular dysfunction with elevated levels among 45% of control subjects, the patients with diabetes had significantly higher levels of Nt-proBNP (Fig. 1). The previously defined cutoff for our assay of 350 pmol/l might either be too sensitive or the true prevalence might be as high as depicted (22,30–36). Further echocardiographical characterization of healthy control subjects with mildly elevated Nt-proBNP levels is warranted.

A linear regression model for age as an independent and Nt-proBNP as a dependent variable suggests an age-related increase in Nt-proBNP of a 4.04-pmol/l

increase per year in the control population. This effect was not seen in the subjects with diabetes (Fig. 2). Several pathophysiological mechanisms might explain why an early deterioration of left ventricular function could overrun the age effect on Nt-proBNP in the patients with diabetes. Hearts from patients with diabetes have an increased collagen content, as have been verified in autopsy studies (5). Another possible mechanism working from the very start of diabetes could be decreased relaxation, and thus diastolic dysfunction, of the myocardium because of ATP deficiency. The intracellular glucose deficiency among patients with diabetes leads to a higher use of free fatty acids through β-oxidation in the myocardium. A sufficient amount of carbohydrate breakdown is of great importance for assuring an adequate function of the ion pumps, meaning Na^+/K^+-ATPase and Ca^{2+}-ATPase, which maintains the right cardiomyocyte membrane potential and intracellular calcium transport that triggers relaxation. In the diabetic heart, this balance is disturbed, proposing a functional explanation to the impaired relaxation in the myocardium (37–40). These effects could be so strong that they would overrun the age effect in the diabetic group. Nt-proBNP has been suggested to be more age sensitive than BNP (41), but in our material, the patients with diabetes show no relation between Nt-proBNP levels and their age. The lack of age effect on the levels of Nt-proBNP seen in patients with diabetes speaks against this supposed disadvantage of Nt-proBNP when it is used for screening in this population. Thus, Nt-proBNP might be especially useful for screening among patients with diabetes (Fig. 2).

Taken together, both BNP and Nt-proBNP serve as sensitive markers of LVD, but the levels of both markers are influenced not only by their rate of synthesis but also their respective clearance rate. The clearance of BNP is more complex through glomerular filtration as well as receptor-dependent mechanisms, whereas Nt-proBNP is cleared solely by glomerular filtration. Because the glomerular filtration rate is easily controlled for, e.g., by cystatin C, and receptor clearance rate cannot be estimated by today's methods, a paired screening with Nt-proBNP and cystatin C might be a preferred screening model in patients with diabetes.

The median Nt-proBNP value in the diabetic group was 360.9 pmol/l as compared with 302.7 pmol/l in the control group. This 58.2-pmol/l difference, according to the Nt-proBNP versus age regression equation in control subjects with a 4.04 pmol/l per year coefficient, would correspond to an age effect of 14.4 years. Rakowski et al. (42) estimated that the E/A value, as a functional measurement of diastolic function, passed 1.0 in patients with diabetes at the age of 56 years as compared with 78 years for the control population.

Blood lipids of traditional risk value differed between groups as expected, with diabetic patients having higher triglycerides and lower HDL cholesterol than control subjects. In addition to the risk of having diabetes, this gives an increased risk of having coronary atheromatosis. However, in our material, diabetic patients did have lower LDL cholesterol. This is probably due to the fact that diabetic subjects were selected to not have any overt cardiovascular disease (Table 1). The testing for Nt-proBNP accompanied with cystatin C is, however, not designed to differ between the atherosclerosis-dependent and -independent decreased cardiac performance. This is a strength rather than a weakness of the assay.

Of all pharmacological compounds tested, Nt-proBNP values did not differ among treated and untreated subjects, with the exception of calcium channel blockers. In the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack study, Ca-channel blockers have been suggested to increase the incidence of heart failure as compared with diuretics. However, the diagnosis of heart failure was not based strictly on scientific definitions in this study but was taken together with our findings. Further studies are warranted to examine this possible relationship (43).

This cross-sectional study has its inherent disadvantages as being only a one-time observation of each individual. Still, the study was able to detect a difference between the two groups. Moreover, this is how it would be used under a screening setting for the individual physician. Even if the groups differ significantly, there is a considerable overlap. Therefore, the test would not be usable for a general screening. However, it could be used as a screening tool to separate patients with diabetes eligible for an echocardiographical examination.

In conclusion, our data suggest that the secretion of Nt-proBNP, if paired with an estimation of glomerular filtration rate, is increased in patients with type 2 diabetes compared with control subjects without overt heart disease. Therefore, measurement of Nt-proBNP paired with cystatin C might be a simple screening tool to identify patients with diabetes at risk for ventricular dysfunction requiring further examination with echocardiography. However, this is a very early study that needs confirmation in larger-scale studies.

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