Arteriosclerosis, Thrombosis, and Vascular Biology

CLINICAL AND POPULATION STUDIES

# Type 2 Diabetes Mellitus Is Independently Associated With Decreased Neural Baroreflex Sensitivity

## The Paris Prospective Study III

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**OBJECTIVE:** Impaired baroreflex function is an early indicator of cardiovascular autonomic imbalance. Patients with type 2 diabetes mellitus (T2D) have decreased baroreflex sensitivity (BRS), however, whether the neural and/or mechanical component of the BRS (nBRS and mBRS, respectively) is altered in those with high metabolic risk (HMR, impaired fasting glucose and/or metabolic syndrome) or with overt T2D, is unknown. We examined this in a community-based observational study, the Paris Prospective Study III (PPS3).

**APPROACH AND RESULTS:** In 7626 adults aged 50 to 75 years, resting nBRS (estimated by low-frequency gain, from carotid distension rate and RR intervals [time intervals between successive R waves]) and mBRS were measured by high-precision carotid echotracking. The associations between overt T2D or HMR as compared with subjects with normal glucose metabolism (NGM) and nBRS or mBRS were quantified using multivariable linear regression analysis. There were 319 subjects with T2D (61±6 years, 77% male), 1450 subjects with HMR (60±6 years, 72% male), and 5857 subjects with NGM (59±6 years, 57% male). Compared with NGM subjects, nBRS was significantly lower in HMR subjects (β=−0.07 [95% CI, −0.12 to −0.01]; *P*=0.029) and in subjects with T2D (β=−0.18 [95% CI, −0.29 to −0.07]; *P*=0.002) after adjustment for confounding and mediating factors. Subgroup analysis suggests significant and independent alteration in mBRS only among HMR patients who had both impaired fasting glucose and metabolic syndrome.

**CONCLUSIONS:** In this community-based study of individuals aged 50 to 75, a graded decrease in nBRS was observed in HMR subjects and patients with overt T2D as compared with NGM subjects.

**VISUAL OVERVIEW:** An online visual overview is available for this article.

**Key Words:** baroreflex ■ blood pressure ■ carotid sinus ■ diabetes mellitus ■ metabolic syndrome ■ vascular stiffness



Arterial baroreflex plays an important role in short-term regulation of blood pressure (BP). Baroreflex sensitivity (BRS) is often used as an estimate of baroreflex function, and impairment of BRS is one of the earliest indicators of cardiovascular autonomic imbalance often undetected by conventional clinical tests.[1] Global BRS is impaired in patients with type 2 diabetes mellitus (T2D)[2] and depressed BRS independently predicts major adverse cardiovascular events in this population.[3]

Traditionally, fluctuations in BP and RR interval (time interval between successive R waves) are used to assess global BRS, which is a combination of both the mechanical (showing the mechanical transduction of BP changes into baroreceptor vessel wall stretch and dependent on the stiffness of the carotid sinus and the aortic arch;

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## Nonstandard Abbreviations and Acronyms

| | |
|---|---|
| **BP** | blood pressure |
| **BRS** | baroreflex sensitivity |
| **HMR** | high metabolic risk |
| **IFG** | impaired fasting glucose |
| **mBRS** | mechanical baroreflex sensitivity |
| **MetS** | metabolic syndrome |
| **nBRS** | neural baroreflex sensitivity |
| **NGM** | normal glucose metabolism |
| **PPb** | brachial pulse pressure |
| **PPS3** | Paris Prospective Study III |
| **RR interval** | time interval between successive R waves |
| **T2D** | type 2 diabetes mellitus |

## Highlights

- In a large community-based study of over 7626 men and women aged 50 to 75, neural baroreflex sensitivity as measured noninvasively by high-precision carotid echotracking decreased linearly across subjects with normal glucose metabolism, subjects with high metabolic risk, and patients with type 2 diabetes mellitus independently from confounding and mediating factors.
- Damage in the mechanical component of baroreflex sensitivity in patients with type 2 diabetes mellitus was due to mediating factors (increased blood pressure, increased heart rate, estimated glomerular filtration rate).
- Independent alteration in the mechanical component of baroreflex sensitivity was observed only in subjects who had both impaired fasting glucose and metabolic syndrome.

mBRS) and neural (reflective of the transduction of baroreceptor stretch into sympathetic/vagal outflow and the cardiac responsiveness; nBRS) components of the baroreflex pathway.[4] Importantly, mBRS and nBRS can be independently altered in several pathologies. For example, increased arterial stiffness can impair baroreflex function in patients with Tetralogy of Fallot,[5] while on the contrary, the deterioration of the neural component is responsible for decreased global BRS in patients with end-stage liver disease.[6] Furthermore, the age-related decline in global BRS is attributable to arterial stiffening and damaged neural control of the baroreflex.[7,8] However, whether the previously observed impairment in global BRS in patients with T2D is due to altered mBRS or nBRS (or both) is not well understood: data regarding mBRS parameters are controversial,[9–11] and alterations in the nBRS have not yet been directly examined. Furthermore, prediabetic states such as metabolic syndrome (MetS) or impaired fasting glucose (IFG) may differentially influence the 2 components.[10,12,13]

The aim of this study was to quantify and compare mBRS and nBRS in subjects with normal glucose metabolism (NGM), with high metabolic risk (HMR) and in patients with T2D at the population level. We hypothesized that there would be a stepwise deterioration in both nBRS and mBRS from NGM towards overt T2D.

# PATIENTS AND METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Study Participants and Overview

This study was a cross-sectional analysis of the PPS3 (Paris Prospective Study III), an ongoing observational prospective study for which the detailed methods can be found elsewhere.[14] Participants provided informed written consent, and the study protocol was approved by the Ethics Committee of the Cochin Hospital (Paris). The study is registered in the international trial registry (URL: http://www.clinicaltrials.gov. Unique identifier: NCT00741728). Briefly, 10 157 volunteers aged 50 to 75 years were recruited from a large preventative medical center, the Centre d'Investigations Préventives et Cliniques in Paris (France) between June 2008 and May 2012. At study recruitment, participants underwent a standard clinical examination, during which resting high-resolution carotid echotracking was performed to measure the components of BRS in a quiet and temperature-controlled room (22±1°C). Participants completed self-administered questionnaires to derive information on lifestyle (ie, physical activity using the validated Baecke questionnaire,[15] diet, smoking, and alcohol consumption) and personal and family medical history. Fasting blood samples were taken to assess standard blood biomarkers.

## Definition of Groups

Glucose metabolism status was determined in line with the current World Health Organization recommendation.[16] NGM was defined as fasting glucose level <110 mg/dL and as the absence of antidiabetic treatment. Subjects with fasting glucose level ≥110 mg/dL and <126 mg/dL and without hypoglycemic medication were diagnosed with IFG. T2D status was defined as fasting glucose level ≥126 mg/dL when untreated or use of oral antidiabetic drugs or insulin. We further subdivided the non-T2D population according to the MetS status. The World Health Organization expert consensus considers MetS a premorbid state; therefore, subjects with established diabetes mellitus were excluded from this category.[17] Subjects with MetS were diagnosed based on the harmonized MetS definition proposed by Alberti et al,[18] and we used it according to the mentioned World Health Organization expert consultation[17] with one modification. We used the cut point 110 mg/dL instead of 100 mg/dL for fasting glycemia to preserve coherency with the aforementioned diagnostic criteria of the disorders of glucose metabolism. Patients having the MetS and/or IFG were grouped

into a HMR group. Detailed information about our MetS criteria can be found in the Data Supplement.

## Carotid Parameters Measurements

All participants fasted for at least 4 hours before carotid echotracking. Subjects rested in supine position for 10 minutes before BP measurement and carotid artery ultrasonography. First, brachial systolic and diastolic BP were measured with an oscillometric method (Omron 705C). Brachial pulse pressure ($PP_b$) was calculated as $PP_b$=systolic BP−diastolic BP and mean BP as diastolic BP+$PP_b$/3. Next, common carotid artery was imaged 2 cm proximal to the carotid bulb in the longitudinal plane using a high-resolution echotracking device (ART. LAB, Esaote, Maastricht, NL) with a conventional ultrasound scanner (7.5 MHz linear array). Common carotid artery external end-diastolic diameter ($D_{ed}$) and intima-media thickness were measured in B-mode (60 Hz, 128 radiofrequency lines), pulsatile distension (ΔD) was measured in fast B-mode (600 Hz, 14 radiofrequency lines). One recording of 6 seconds was made both in B-mode and in fast B-mode. Then, one long-term recording of the common carotid artery was performed over 5 minutes in fast B-mode. Carotid pulse pressure was determined using the calibration of carotid distension waveforms registered by echotracking as reported by Van Bortel et al.[19] This procedure is based on the fact that the difference between mean BP and diastolic BP is constant throughout the large artery tree. Carotid pulse pressure ($PP_c$) is calculated from $PP_b$ and the K factor at the carotid and the brachial arteries ($K_c$ and $K_b$, respectively) as follows: $PP_c=PP_b\times K_c/K_b$. $K_c$ is defined as $(D-D_{ed})/\Delta D$, where D is the mean external diameter calculated by dividing the area under the distension wave by time. The calculation of the $K_b$ is (mean BP−diastolic BP)/$PP_b$.

## Mechanical BRS

Carotid stiffness representing mBRS was calculated using the Bramwell-Hill equation as follows: $\text{mBRS} = \text{Carotid stiffness} = \sqrt{1/(\rho \times DC)}$, where ρ is the density of blood, and DC is the distensibility coefficient of the carotid artery.[20] DC shows the relative change in lumen area during systole for a given pressure change and is calculated as follows: $DC=\Delta A/(A\times PP_c)$, where A is end-diastolic lumen cross-sectional area, and ΔA is the change in lumen area during systole. The mBRS shows the local carotid pulse wave velocity in meters per second (m/s). It is a widely accepted and used marker of local arterial stiffness.[11,12,20] Please note that elevated values of mBRS indicate altered function. Other elastic parameters of the carotid artery that represent other metrics of the mechanical component of BRS were also calculated (material in the Data Supplement).

## Neural BRS

RR intervals were derived from the time difference between marks placed on the foot of the carotid diameter curve over the 5 minutes time period acquired at 600 Hz. The nBRS was calculated as reported earlier.[21] Briefly, the common carotid artery distension rate was defined as the distension change between 10% and 90% of the systolic rise divided by the associated rise-time. Simultaneous beat-to-beat carotid distension, distension rate, and RR interval were acquired for at least 300 seconds. A section of 256 heart beats was selected for analysis. Power spectra of distension rate and RR interval were obtained by Fast Fourier transformation. Since the relationship between the variability of the stimulus parameter and the variability of RR intervals shows baroreflex origin in the low-frequency band[22,23] mean cross-spectral transfer gain between distension rate and RR interval signals in the frequency band of 0.04 to 0.15 Hz defined the low-frequency gain and represented nBRS. Resting heart rate was also derived from the 5-minute-long fast B-mode recording as follows: heart rate (beats/min [bpm])=(60 [s/min])/(mean RR interval [s/beat]).

## Statistical Analysis

Statistics were performed with SAS software 9.4 (Statistical Analysis System, Cary, NC). Data with normal distribution are expressed as mean±SD. Variables with skewed distribution (fasting glucose and triglycerides) were logarithmically transformed and are presented as median (interquartile range). Low-frequency gain was ln-transformed as follows: nBRS, normalized units=ln ($10^2\times$low-frequency gain). Unadjusted test for trend across the groups using Armitage $\chi^2$ or linear regression for categorical and continuous variables respectively were employed. Multivariable linear regression with Tukey post hoc test was used to quantify the associations between the subject groups and the arterial parameters. The association of HMR/T2D with nBRS and mBRS was first adjusted for potential confounders (age, sex, BMI, smoking, alcohol consumption, and physical activity score). Further adjustments were made for suspected mediators identified from the literature (mean BP, estimated glomerular filtration rate, statin use, and additionally, mBRS in the case of nBRS, heart rate in the case of mBRS—we did not adjust for heart rate when investigating nBRS due to potential collinearity). To assess the separate influence of abnormal glucose levels and other metabolic disturbances, the HMR group was split into the 3 following subgroups: IFG without MetS, MetS without IFG, and MetS with IFG. The analysis for these 3 subgroups, in addition to NGM and T2D, was adjusted for confounders and mediators. Several sensitivity analyses were conducted to assess the robustness of our findings. First, subjects under insulin treatment (potentially having type 1 diabetes mellitus) were excluded. Second, to address residual confounding by antihypertensive medication, analysis was first adjusted for antihypertensive medication (yes/no) and then for antihypertensive drugs (β-blocking agents; calcium channel blockers; agents acting on the renin-angiotensin system; diuretics and other antihypertensive agents). Third, analyses were repeated using compliance coefficient, distensibility coefficient and Young's elastic modulus representing other metrics of the mechanical component of BRS. Last, to ease international comparison with other studies, analyses were only adjusted for age, sex, and mean BP.[9,11]

In all analyses, the continuous variables were included in the final models in standardized forms using *z* scores. The threshold for statistical significance was $P<0.05$.

# RESULTS

## Study Population

Figure I in the Data Supplement shows the selection and categorization of the study population. Of the initial

10 157 recruited participants, 2321 had missing data on carotid echotracking parameters and covariates. We additionally excluded subjects with prior cardiovascular diseases (n=210) to eliminate the potential confounding influence on mBRS and nBRS. Compared with included participants, those who were excluded had higher body mass index and BP and were more likely to smoke and take lipid-lowering medication (Table I in the Data Supplement). Our study population (n=7626) consisted of 3 groups: subjects with NGM (n=5857), subjects with HMR (IFG and/or MetS; n=1450), and patients with T2D (n=319). Their baseline characteristics are shown in Table 1. The mean age was 60 years, and 40% of the whole population were women. Patients with HMR and those with T2D had significantly higher body mass index, BP and heart rate, were more likely to be men, take BP and lipid-lowering medication and have less favourable biochemical profile compared with the subjects with NGM. Furthermore, nBRS decreased and mBRS increased significantly across the groups. The results were similar when other carotid elastic parameters were examined (Table II in the Data Supplement).

## Multivariable Associations Between HMR and T2D With nBRS and mBRS

The regression coefficients and 95% CI of the multivariable association of HMR and T2D as compared with NGM with nBRS and mBRS are reported in Table 2, while the regression coefficients and 95% CI of the covariates are reported in Tables III and IV in the Data Supplement. After adjusting for the confounding factors and compared with NGM subjects, nBRS was significantly lower in T2D whereas the association was borderline significant in HMR subjects. Furthermore, mBRS was significantly lower in both HMR and T2D subjects as compared with NGM subjects. After additional adjustment for the mediating factors, nBRS was significantly lower in HMR subjects and in subjects with T2D. Instead, the association between HMR or T2D with mBRS was no longer significant. In these models, in addition to HMR status and T2D, age, sex, body mass index, smoking, physical activity score (confounding factors), mean BP, estimated glomerular filtration rate, and mBRS (mediating factors) were significantly associated with nBRS. Factors significantly associated

**Table 1. Participant Characteristics**

| | NGM (n=5857) | HMR (n=1450) | T2D (n=319) | *P* Trend |
|---|---|---|---|---|
| Age, y | 59±6 | 60±6* | 61±6*† | <0.0001 |
| Male, n (%) | 3311 (57) | 1038 (72)* | 247 (77)* | <0.0001 |
| Body mass index, kg/m$^2$ | 24.40±3.32 | 27.12±3.64* | 27.70±4.14*† | <0.0001 |
| Waist circumference, cm | 84.1±11.0 | 92.8±10.9* | 95.3±10.9*† | <0.0001 |
| Current smoker, n (%) | 832 (14) | 228 (16) | 39 (12) | 0.75 |
| Consume alcohol, n (%) | 5167 (88) | 1289 (89) | 263 (82)*† | 0.101 |
| Total physical activity | 6.9±1.5 | 6.8±1.6* | 6.6±1.6* | <0.0001 |
| Systolic BP, mm Hg | 129±16 | 136±15* | 137±16* | <0.0001 |
| Diastolic BP, mm Hg | 75±9 | 79±10* | 78±10* | <0.0001 |
| Mean BP, mm Hg | 93±11 | 98±10* | 98±10* | <0.0001 |
| Resting heart rate, bpm‡ | 68±10 | 71±12* | 73±13*† | <0.0001 |
| BP lowering medication, n (%) | 710 (12) | 343 (24)* | 136 (43)*† | <0.0001 |
| Lipid lowering medication, n (%) | 560 (10) | 306 (21)* | 103 (32)*† | <0.0001 |
| Glucose lowering medication, n (%) | … | … | 169 (53) | … |
| Fasting glucose, mg/dL§ | 97 (92–102) | 110 (101–114)* | 132 (120–148)*† | <0.0001 |
| Total cholesterol, mg/dL | 221.3±34.6 | 225.6±36.2* | 206.5±44.0*† | 0.0260 |
| HDL cholesterol, mg/dL | 61.0±14.9 | 51.5±14.2* | 51.2±13.9* | <0.0001 |
| LDL cholesterol, mg/dL | 142.1±30.8 | 147.2±32.1* | 129.7±38.3*† | 0.34 |
| Triglycerides, mg/dL§ | 83 (66–107) | 125 (86–169)* | 113 (87–158)* | <0.0001 |
| eGFR, mL min$^{-1}$ 1.73 m$^{-2}$ | 79.11±12.71 | 77.43±13.19* | 78.27±13.31 | 0.0002 |
| nBRS, NU | 2.96±0.63 | 2.89±0.63* | 2.80±0.67* | <0.0001 |
| mBRS, m/s | 7.0±1.3 | 7.4±1.4* | 7.6±1.4* | <0.0001 |

Data are mean±SD unless otherwise stated. BP indicates blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HMR, high metabolic risk; LDL, low-density lipoprotein; mBRS, mechanical baroreflex sensitivity; nBRS, neural baroreflex sensitivity; NGM, normal glucose metabolism; and T2D, type 2 diabetes mellitus.

*Significant difference compared with subjects with NGM.

†Significant difference compared with subjects with HMR.

‡Resting heart rate was derived from the 5-minute-long fast B-mode recording.

§Data are median (interquartile range).

**Table 2. Multivariable Association Between High Metabolic Risk (n=1450) or Type 2 Diabetes Mellitus (n=319) With Neural Baroreflex Sensitivity and Mechanical Baroreflex Sensitivity as Compared With Normal Glucose Metabolism (n=5857)**

| | nBRS | mBRS |
|---|---|---|
| Adjusted for confounding factors* | | |
| Normal glucose metabolism | Ref | Ref |
| High metabolic risk | −0.06 (−0.12 to 0.00), $P$=0.059 | 0.17 (0.11 to 0.23), $P$<0.0001 |
| Type 2 diabetes mellitus | −0.16 (−0.28 to −0.05), $P$=0.006 | 0.20 (0.09 to 0.31), $P$=0.0003 |
| Additionally adjusted for mediating factors† | | |
| Normal glucose metabolism | Ref | Ref |
| High metabolic risk | −0.07 (−0.12 to −0.01), $P$=0.029 | 0.04 (−0.01 to 0.10), $P$=0.12 |
| Type 2 diabetes mellitus | −0.18 (−0.29 to −0.07), $P$=0.002 | 0.08 (−0.02 to 0.18), $P$=0.12 |

Data are unstandardized regression coefficients and 95% CIs. The continuous variables were included in the models in standardized forms using *z* scores. mBRS indicates mechanical baroreflex sensitivity; and nBRS, neural baroreflex sensitivity.

*Confounding factors: age, sex, body mass index, smoking, alcohol consumption, physical activity score.

†Mediating factors: mean blood pressure, estimated glomerular filtration rate, statin therapy; mBRS in the case of nBRS; heart rate in the case of mBRS but not in the case of nBRS.

with mBRS were age, sex, body mass index, alcohol consumption (confounding factors), mean BP, heart rate, and estimated glomerular filtration rate (mediating factors; Table IV in the Data Supplement).

Subgroup analysis (Table 3) further indicates that the lower nBRS in HMR subjects as compared with the NGM subjects was observed in HMR subjects with MetS and in HMR subjects with both MetS and IFG but not in HMR subjects with IFG alone. In addition, the altered mBRS in HMR subjects as compared with the NGM subjects was observed only in those who had both the MetS and IFG.

## Sensitivity Analysis

First, exclusion of patients treated by insulin did not change the main results (Table V in the Data Supplement). Second, further adjustment for antihypertensive treatment (yes/no) and for antihypertensive medication classes showed essentially unaltered results (Tables VI and VII in the Data Supplement). Third, similar results were observed when other metrics of carotid stiffness (compliance coefficient, distensibility coefficient, and Young's elastic modulus) were used (Table VIII in the Data Supplement). Last, when analyses were adjusted only for age, sex, and MBP (Figure), nBRS decreased

**Table 3. Multivariable Association Between Subgroups of High Metabolic Risk (n=1450) or Type 2 Diabetes Mellitus (n=319) With Neural Baroreflex Sensitivity and Mechanical Baroreflex Sensitivity as Compared With Normal Glucose Metabolism (n=5857)**

| | nBRS | mBRS |
|---|---|---|
| Normal glucose metabolism | Ref | Ref |
| IFG, no MetS (n=420) | 0.05 (−0.05 to 0.14); $P$=0.33 | −0.06 (−0.15 to 0.03); $P$=0.17 |
| MetS without IFG (n=624) | −0.10 (−0.18 to −0.02); $P$=0.019 | 0.06 (−0.02 to 0.14); $P$=0.11 |
| MetS with IFG (n=406) | −0.15 (−0.25 to −0.05); $P$=0.004 | 0.14 (0.05 to 0.24); $P$=0.002 |
| Type 2 diabetes mellitus | −0.18 (−0.30 to −0.07); $P$=0.001 | 0.09 (−0.02 to 0.19); $P$=0.095 |
| Age | −0.16 (−0.19 to −0.14); $P$<0.0001 | 0.21 (0.19 to 0.24); $P$<0.0001 |
| Sex | 0.08 (0.04 to 0.13); $P$=0.0007 | −0.06 (−0.10 to −0.01); $P$=0.012 |
| Body mass index | −0.06 (−0.09 to −0.04); $P$<0.0001 | 0.10 (0.08 to 0.12); $P$<0.0001 |
| Smoking | −0.11 (−0.17 to −0.04); $P$=0.001 | −0.01 (−0.06 to 0.05); $P$=0.87 |
| Alcohol consumption | 0.05 (−0.02 to 0.12); $P$=0.16 | −0.08 (−0.14 to −0.02); $P$=0.016 |
| Physical activity score | 0.03 (0.01 to 0.05); $P$=0.020 | 0.00 (−0.03 to 0.02); $P$=0.75 |
| Mean blood pressure | −0.14 (−0.16 to −0.11); $P$<0.0001 | 0.30 (0.28 to 0.32); $P$<0.0001 |
| Heart rate | … | 0.11 (0.09 to 0.14); $P$<0.0001 |
| Statin use | −0.04 (−0.12 to 0.03); $P$=0.27 | −0.03 (−0.10 to 0.04); $P$=0.43 |
| eGFR | −0.05 (−0.07 to −0.03); $P$<0.0001 | −0.07 (−0.09 to −0.04); $P$<0.0001 |
| mBRS | 0.25 (0.23 to 0.28); $P$<0.0001 | … |

Data are unstandardized regression coefficients and 95% CIs. The continuous variables were included in the models in standardized forms using *z*-scores. Analysis was adjusted for the variables included in the table. eGFR indicates estimated glomerular filtration rate; IFG, impaired fasting glucose; mBRS, mechanical baroreflex sensitivity; MetS, metabolic syndrome; and nBRS, neural baroreflex sensitivity.

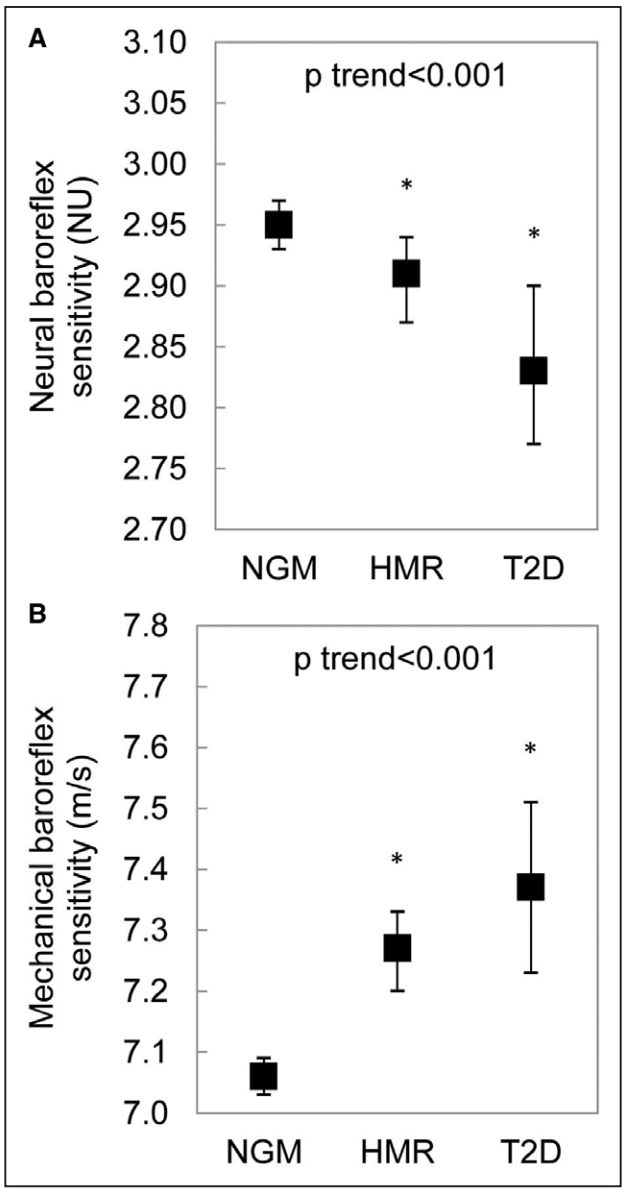


**Figure. Distribution of baroreflex sensitivity components in subjects with normal glucose metabolism (NGM), subjects with high metabolic risk (HMR), and patients with type 2 diabetes mellitus (T2D).**
Neural baroreflex sensitivity (**A**), mechanical baroreflex sensitivity (**B**). Mean values and 95% CIs are adjusted for age, sex, and mean blood pressure. * indicates statistically significant difference compared with subjects with NGM.

while mBRS increased linearly across the 3 groups (*P* for trend<0.001 for both nBRS and mBRS).

## DISCUSSION

In this large study of community-dwelling adults aged 50 to 75, nBRS was significantly and gradually lower in patients with HMR and in those with overt T2D compared with subjects with NGM independently from confounding and mediating factors. Impairment of mBRS in the T2D group as compared with subjects with NGM was explained by mediating factors such as increased BP, increased heart rate, and estimated glomerular filtration rate. Alteration in mBRS in subjects with HMR as compared with subjects with NGM was seen in those with both IFG and MetS in subgroup analysis.

Only a few previous studies have investigated nBRS alteration in patients with diabetes mellitus. Ruiz et al[2] demonstrated that neuropathy measured at the periphery is a more important determinant of global BRS than carotid distensibility in patients with T2D. Lipponen et al[24] also showed impaired nBRS in a small group of patients (n=15) with type 1 diabetes mellitus using methods similar to ours. However, compared with this earlier work, we have shown in a much larger sample size and using a method specifically developed for investigating the neural and mechanical components of the BRS separately, that nBRS is impaired in patients with T2D. Our results are obtained at a population level, with patients having milder presentation of the disease. The results of this study underline the importance of lifestyle-modification and treatment development in patients with T2D because the therapeutic repertoire for improving cardiovagal neural activity is imperfect. Enhanced glucose control elicits only a modest reduction in neuropathy in patients with T2D[25–27] in contrast to the considerable effect in patients with type 1 diabetes mellitus.[28] Based on our results and recent results of another substudy of PPS3,[29] regular physical activity could play an important role in the lifestyle-modification process. Beside other favorable effects exercise training improves global BRS in patients with T2D[30] and ameliorates nBRS even at advanced ages.[31] In line with earlier findings, we observed a negative association between elevated BP and baroreflex function[32] and a similar relationship for smoking.[33] Accordingly, multifactorial intervention should be applied to prevent further damage of neural structures. As the Steno-2 study showed, progression of autonomic neuropathy profoundly decreased in the T2D group where an intensive multifactorial therapeutic approach was used with strict treatment goals in reference to glycemic control, weight control, control of BP, cessation of smoking, encouragement for performing more physical exercise and other interventions.[34] This beneficial effect of the 7.8 years intensified treatment regarding autonomic neuropathy was still observable after 21.2 years of follow-up.[35] Similar results were found by Gibbons et al[36] when there was no observable progression in cardiovascular autonomic dysfunction over a 3-year-long period in patients with well-controlled risk factors and T2D. Furthermore, our results reveal the importance of treatment development that is based on pathogenic concepts. Additionally, the recognition of early neural damage without any symptoms could overcome clinical inertia and elicit a proactive behavior in noncompliant patients.[37]

There are a limited number of studies focusing on the nBRS in prediabetic states. In a substudy of the

PPS3 (n=2835), Zanoli et al[12] found decreased nBRS in patients with MetS. The difference in nBRS between HMR subjects (ie, with IFG and/or MetS) and NGM subjects was also significant in the present analysis after adjustment for confounders and mediators. In line with the previous substudy, our subgroup analysis confirmed that the significant and independent difference was mediated by the accumulation of metabolic disturbances that define MetS and less by abnormal glucose level per se. Our results are in line with the findings of Wu et al[38] who showed that the state of IFG is not independently associated with baroreflex impairment.

Earlier results regarding the association between carotid elasticity representing mBRS and T2D are controversial. While the Hoorn study and the Maastricht study showed independent association between carotid stiffening and T2D status,[9,11] the Asklepios study did not show a similar relationship.[10] One explanation for the lack of independent association between T2D and mBRS in our study is that the vast majority of the patients included in the current study were in the very early stage of diabetes mellitus with only 8 patients treated with insulin in our population. We also excluded patients with prior cardiovascular diseases to avoid the possible influence on the components of BRS. A further explanation of our results could be the voluntary participation of the subjects in the PPS3 that could lead to a relatively health-oriented population. In line with these assumptions, only 4.3% of the PPS3 participants had T2D, which is much less than the age-specific prevalence of T2D in France.[39–41] Last, there is a 25 years difference (from age 50–75) in the constitution of the cohort, and population characteristics might be notably different (sensitivity to risk factors, exposure to different treatments, evolution of socioeconomic context etc). Taken together, early stage and good clinical control could explain our results showing that impaired carotid elastic function is not an intrinsic phenomenon of T2D in our population; instead, it is explained by mediating factors like increased BP, increased heart rate or renal function. Therefore, the main focus of therapy and future research should target these factors. Elevated heart rate could be the consequence of decreased nBRS in our T2D group. Although the underlying mechanism of the stiffening action of elevated heart rate is not entirely clear,[42] improvement of neural functions could also have beneficial effects on mBRS through the lowering of baseline heart rate.

Similar to T2D, studies examining carotid elastic parameters in prediabetic conditions showed controversial results.[9,10,12,13,43–48] However, since diagnostic criteria of prediabetic states were different in these studies, it is hard to make clear conclusions regarding the elastic function of the carotid artery in patients with HMR. We showed that stiffening of the carotid artery is already present before overt T2D and that is likely due to mediating factors. In agreement with the results of the Rotterdam study, we did not find an independent association between altered elastic function and IFG in subjects younger than 75 years.[13] In contrast, we observed that subjects with the simultaneous presence of MetS and IFG had significantly higher mBRS compared with the NGM group. This result could partially explain the findings of Guize et al.[49] They examined the risk of short-term all-cause mortality in different component combinations of MetS. They found that those 3-component combinations that were associated with higher risk of all-cause mortality included the component of elevated glucose level in the majority of the cases. This finding is also in line with the results of the MARE Consortium.[50] They measured carotid-femoral pulse wave velocity in different clusters of MetS components. They showed that the majority of the clusters of MetS components that were associated with extremely stiff arteries included the component of elevated glucose level.

Statin therapy or treatment with different classes of antihypertensive drugs did not have substantial influence on our main results. We did not make adjustment for antidiabetic treatment to avoid substantial overfitting of our models. The literature about the effects of antidiabetic medication on baroreflex function is limited. Metformin was related to improved baroreflex function in previous animal experiments.[51,52] Recent results of the Maastricht study showed that use of metformin was not associated with lower carotid stiffness.[53]

Our study had several strengths. We included data from a large, well-characterized study sample and used highly specialized and sensitive technique to measure nBRS and mBRS at the same site. BRS is traditionally measured using RR interval responses to changes in systolic BP measured at the periphery. However, peripheral BP values may not properly represent the pressure at the level of the baroreceptors due to wave propagation and wave reflection.[54] Therefore, we measured local (carotid) BP, carotid diameter and distension to calculate mBRS and examined the spectral relationship between carotid distension rate and RR interval signals to estimate nBRS. Accordingly, we received more detailed information about baroreflex function without the confounding effect of wave propagation and wave reflection which is influenced by the mechanical properties of peripheral arteries.[54] However, there are some limitations that should be considered. T2D diagnosis was only based on a fasting blood glucose measure and hemoglobin $A_{1c}$ level measurement or oral glucose tolerance test to confirm the presence of diabetes mellitus were not performed in the current study. We were not able to distinguish between type 1 and T2D, however, since the main results did not change after the exclusion of patients treated with insulin (suspected to have type 1 diabetes mellitus; n=8) we believe that the potential presence of a few patients with type 1 diabetes mellitus did not lead to draw false conclusions. The cross-sectional nature of our study limits inference regarding causality. The relationship between IFG, MetS, and BRS is based on subgroup analysis and should, therefore, be

interpreted with caution. Finally, the study was conducted in a predominantly white population and our results should be examined in more ethnically diverse populations.

## CONCLUSIONS

This study provides a systematic comparison of neural and mechanical components of the BRS between subjects with NGM, subjects with HMR and patients with T2D. We observed a graded decrease in nBRS across NGM, HMR, and T2D that was independent from confounding and mediating factors. Subgroup analysis suggests significant and independent alteration in mBRS only in HMR subjects with both IFG and the MetS.

## ARTICLE INFORMATION

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### Disclosures

None.

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